NVRI SEMINAR SERIES 2015

This seminar series is a collation of seminar papers presented by staff and visiting scientists to the National Veterinary Research Institute, Vom during 2015

Compiled and edited by

NVRI Seminar/Publication Committee 2015

Dr P. A. Okewole . . .	Chairman
Dr J. Kamani . . .	Member
Dr I. S. Tekki . . .	Member
Dr A. T. Oladokun . .	Member
Dr W. Bertu . . .	Member
Dr (Mrs.) V. Ifende. . . .	Member
Dr O. O. Oladipo . . .	Member
Dr I. J. Barde . . .	Member
Mr. T. Barko . . .	Member
Mr. J. U. Ogbu . . .	Member
Mr. Jerry Musa . . .	Member
Dr (Mrs.) C. I. Odita . .	Secretary
Mr. Francis Dama. . .	Assistant Secretary

National Veterinary Research Institute, Vom

PMB 01, Vom

Email edvr@nvri.gov.ng or nvri1924@yahoo.com

Website: www.nvri.gov.ng

ISSN1597-1651

TABLE OF CONTENTS

INTRODUCTION

Seminar presentation in the Institute is accorded priority in recognition of the fact that any information that is not shared is knowledge lost. Current trends and cutting edge research methodologies were presented by staff of the Institute and Visiting Scientist in order to open up new vistas and broaden the research horizon of other researchers and staff. A blend of topical research and diagnostic procedures, current concepts in administration and psychological assessment of job performance were aptly presented to savor the appetite of all cadres of staff.

In this compilation, "NVRI Seminar Series 2015", we invite you to read through the collation of seminars presented in the year. Papers presented cover diverse areas of research in Veterinary Medicine, Molecular Biology, Microbiology, Pathology and Genetic Engineering, etc. The socio economic effect of parasitic infection to man and animals and the need to curb their menace were presented. This fact is being buttressed by the high prevalence of helminths infection in cattle in Kachia Grazing Reserve and molecular detection and characterization of *Trypanosoma congolense* and *T. brucei* in cattle in Plateau state. Overall, a multi faceted approach to the control of parasitic diseases was considered. They include the use of modeling to map the risk and predict intervention strategy for haemonchosis, the use of bio active substances to control lymnae snails as well as exploiting the synergistic/potentiating effects of *Azadirachta indica* and *Khaya senegalensis* in the control of avian coccidiosis.

The role of Salmonellae as a major pathogen of birds and livestock in Nigeria was elucidated in several of the papers presented. Several serovars were isolated and characterized from hatcheries, poultry farms and cattle indicating widespread circulation of this bacterium in the environment and between different animal species. Similarly, an experimental pathogenicity test of *Pasteurella multocida* in chicken, quails and mice revealed that the organism is capable of inducing high mortality in susceptible stock. The attention of researchers was drawn to the use of Bruce-ladder Multiplex PCR for the characterization of *Brucella* isolates, the organism which was reported to be endemic in the areas studied.

Serological evidence of the circulation of Swine Influenza A virus in pigs in Jos South LGA was presented with the possible role of pigs as a mixing vessel highlighted. Could this be linked to the emergence of a new clade of Avian

Influenza virus as the etiology of the current outbreaks in poultry in 20 states and FCT. Read through these papers and adjudicate for yourself.

Vaccine producers were cautioned on the contaminative role of endogenous retrovirus in feline cell lines routinely used for vaccine production and the possible long term effect on vaccinated animals. New research areas such as Antibody engineering as a research tool and the use of reverse genetics in functional genomics are featured in this series.

It is my pleasure to usher you to read this document for the dissemination and advancement of knowledge for improved agricultural research and livestock and human health.

Dr. P. A. Okewole

Chairman,

Seminar and Publications Committee

MODELLING THE RISK OF HAEMONCHOSIS IN SHEEP

Bolajoko Muhammad-Bashir[1*], Eric Morgan[2]

[1] Epidemiology Unit, NVRI, Vom, Plateau, Nigeria

[2] University of Bristol Veterinary School, c/o Bristol Life Sciences Building, 24 Tyndall Avenue, Bristol BS8 1TQ

INTRODUCTION

Of all the diseases caused by the gastrointestinal nematodes of small ruminants, haemonchosis is perhaps one of the most common and serious diseases in the tropics and summer dominant rainfall regions of the world (O'Connor *et al.*, 2006).

Eggs and larval development of *H. contortus* is optimal in warm and humid conditions; as such temperature and moisture are the dominant influences on the free-living larval stages, with other factors often controlling these two at the microclimate level (O'Connor et al., 2006). It is therefore agreed that temperature and moisture alone determine the success rate and speed of development, both of which predict the epidemiology and ecology of *H. contortus* (Tembely *et al.*, 1997). Studies conducted by various researchers around the globe confirm that knowledge of local climate and how it drives seasonal and shorter-term patterns in *L3* availability is therefore crucial to ability to predict and manage infection and disease threat (Rose, 1963).

Grazing animals pick up infective larvae on herbage that is relatively short or from herbage tops (Silva, *et al.*, 2008). Outbreaks are worst with sudden increased pasture larval density when warm summer rains follow a period of drought break up the faecal pellet and create a moist environment for the migration of hatched *L3* (Silva, *et al.*, 2008). When moisture is not a limiting factor, temperature asserts a greater influence over migration (Stromberg, 1997).

Mathematical modelling has received increasingly wide application in ecological and epidemiological studies of infectious diseases. This is the result of increased understanding of what models can offer in terms of prediction and understanding of a disease process (Smith and Grenfell, 1994). In epidemiology and ecology, models permit the prediction of disease

dynamics at the level of the entire population from the understanding of epidemiological factors at the individual level (Keeling and Rohani, 2008). Modelling also enables the design and experimental evaluation of the impact of specific management practices or control measures based on the predicted parasite dynamics (Roberts and Heesterbeck, 1995). Further work needs to be done on how modelling can be adapted as a tool for predicting risk in practice (Kahn, 2010), and to guide targeted selective treatment (TST) and targeted treatment (TT) as part of holistic and sustainable control against *H. contortus.*

The objectives of this study are to develop and validate a predictive mechanistic model of haemonchosis in sheep by verifying and comparing model predictions with either sourced or on-farm data on haemonchosis, as well as to map the geographic distribution of haemonchosis risk.

MATERIALS AND METHODS

Threshold quantities

Threshold quantity is defined as the average number of adult parasites produced by one female parasite in the absence of density dependent constraints during the entire reproductive lifespan when introduced into a previously unexposed host population (Heesterbeek and Roberts, 1995).

Basic reproduction rate (Q_0)

As a threshold quantity, Q_0 should be able to identify and define conditions or factors that affect the parasite and host population (Scott and Smith, 1994). This is very critical to sustainable control of *H. contortus.* A large value of Q_0 indicates that a nematode is relatively well adapted to a given climate and region, which implies stronger tendency towards population growth in the absence of immunity resulting in higher infection pressure (Kao *et al.*, 2000).

Assumptions on which the present Q_0 model was built

- Q_0 estimates the mean output of adult progeny of an adult worm in a non immune host without any density dependent constraint.
- infective larvae are evenly spread over pasture (Kao *et al.*, 2000);

- development, mortality and transmission rates of all free-living stages are dependent on the prevailing microclimate at any given point in time (Kao *et al.*, 2000);

The Q_0 model

The Q_0 model is a combination of four components.

Term 1 (fecundity rate): This measures the output during the entire reproductive lifetime of the adult female within the host.

Mathematical representation of term 1 = $\lambda / 2\mu p$..... *(1)*

λ is a measure of fecundity of female in terms of egg production rate and μp is the mortality rate of the parasite where the 2 represent the contribution of both the male and female to the whole process.

Term 2 (probability of development to infective larvae as well as extra-pellet migration): This component estimates the chance that eggs being deposited by sheep on the pasture will successfully develop and yield *L3,* followed by successful migration of *L3* out of faeces unto pasture.

The second term is measured using $d_e d_h / (\mu_e + d_e)(\mu L_3 + d_h)$..... *(2)*

Where d_e is development rate from egg to *L3,* d_h is the migration rate of *L3* from the ground onto herbage, μ_e is the mortality rate of egg and μL_3 is the mortality rate of *L3* on ground. The combination of the success of both d_e and d_h is a measure of infective potential of *Haemonchus contortus.*

Term 3 (probability of ingestion of L3 on herbage): This component is built to estimate the probability of actual transmission of *H. contortus* to the host via grazing.

The third term is measured using the formula = $cH / (bA\mu L_h + cH)$.....*(3)*

The mortality rate of L_h (μL_h), host stocking density (H/A) , standing biomass (b) , grazing area (A) and daily larval ingestion rate per sheep per grazing area (c) are all factors that have contributory effects on the overall chance of successful transmission of L_h to the host via ingestion.

Term 4 (probability of establishment): The fourth component measures the probability of establishment of *L3* following ingestion by the host.

The fourth term is represented by the symbol = *pe..... (4)*

Putting the terms together:

$$Q_0 = \lambda / 2\mu p \ * \ d_e d_h / \ (\mu_e + d_e)(\mu L_3 + d_h) * cH/(bA\mu L_h + cH) * pe$$

(Fecundity) (Chance of development) (Chance of ingestion) (Chance of establishment)

The Q_0 model is deterministic in foundation and structure with windows to incorporate stochastic elements via imputation of parameterizable range of parameters dependent on prevailing climatic conditions.

Data

Climatic data to run the model were sourced from the unpublished data of the Met Office for the UK. Haemonchosis data for model validation were sourced from the unpublished UK veterinary laboratory data (Veterinary Investigation Diagnosis Analysis database (VIDA). A 25km gridded monthly dataset for the whole of the UK for both temperature and rainfall was provided from 2001 – 2006 by the UK Met Office (UKCP09, which is available on request via website: http://ukclimateprojections.metoffice.gov.uk/).

Spatial database creation

A spatial database constituting a single layer of Q_0 predictions was created for UK. 10 (ESRI, Redlands, CA) was used for the introduction and cartography of the basic reproduction rate model predictions as layer into the GIS.

RESULTS

Regional variation in haemonchosis risk across UK, 2001 to 2006

The result of the Pearson correlation test between the variations in the average haemonchosis risk and average reported haemonchosis incidence across all regions of the UK, from 2001 to 2006 (Fig 1) is 0.68, ($P<0.05$).

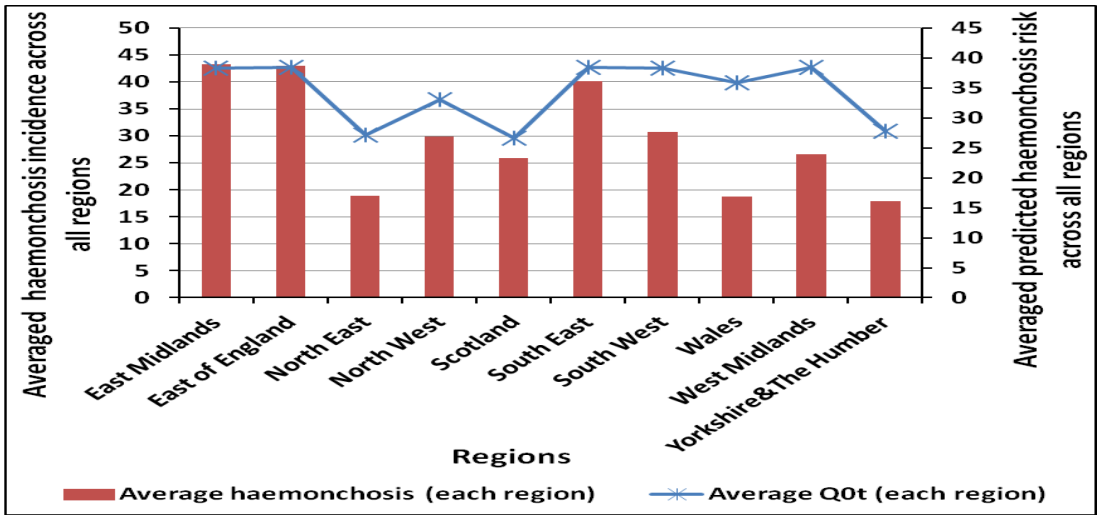


Figure 1: Comparison between the variations in the averaged haemonchosis incidence reported with that of the averaged predicted haemonchosis risk across all regions of the UK, from 2001 to 2006**.**

Seasonal variation in haemonchosis risk across the UK, 2001 – 2006

The results of the Pearson correlation between the average monthly predictions of haemonchosis risk and the total monthly haemonchosis incidence reported across the UK (Fig. 2) = 0.93, (P<0.01).

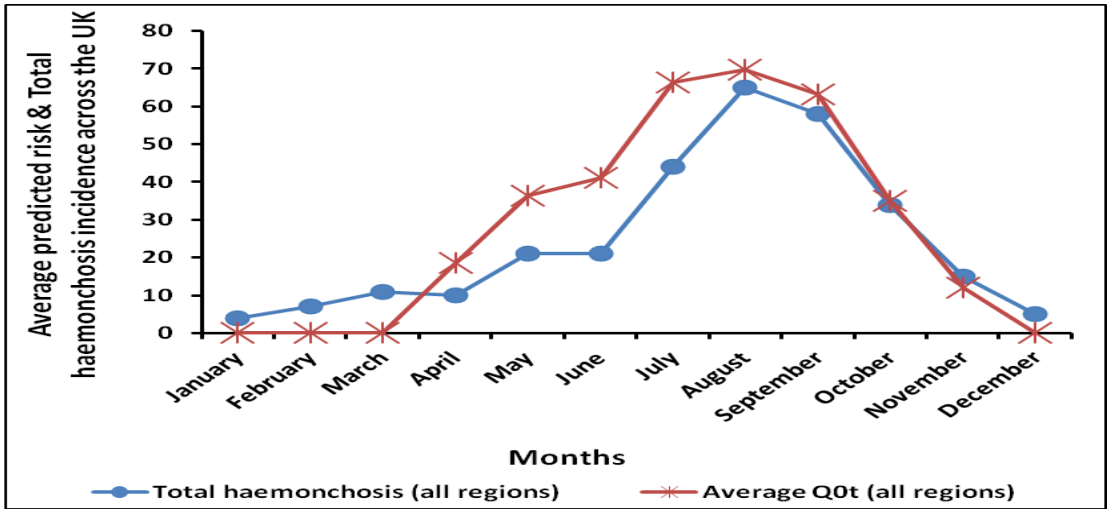


Figure 2: A comparison between the seasonal variations in the average Q_0 predicted haemonchosis risk and the total haemonchosis incidence across the UK, 2001 to 2006.

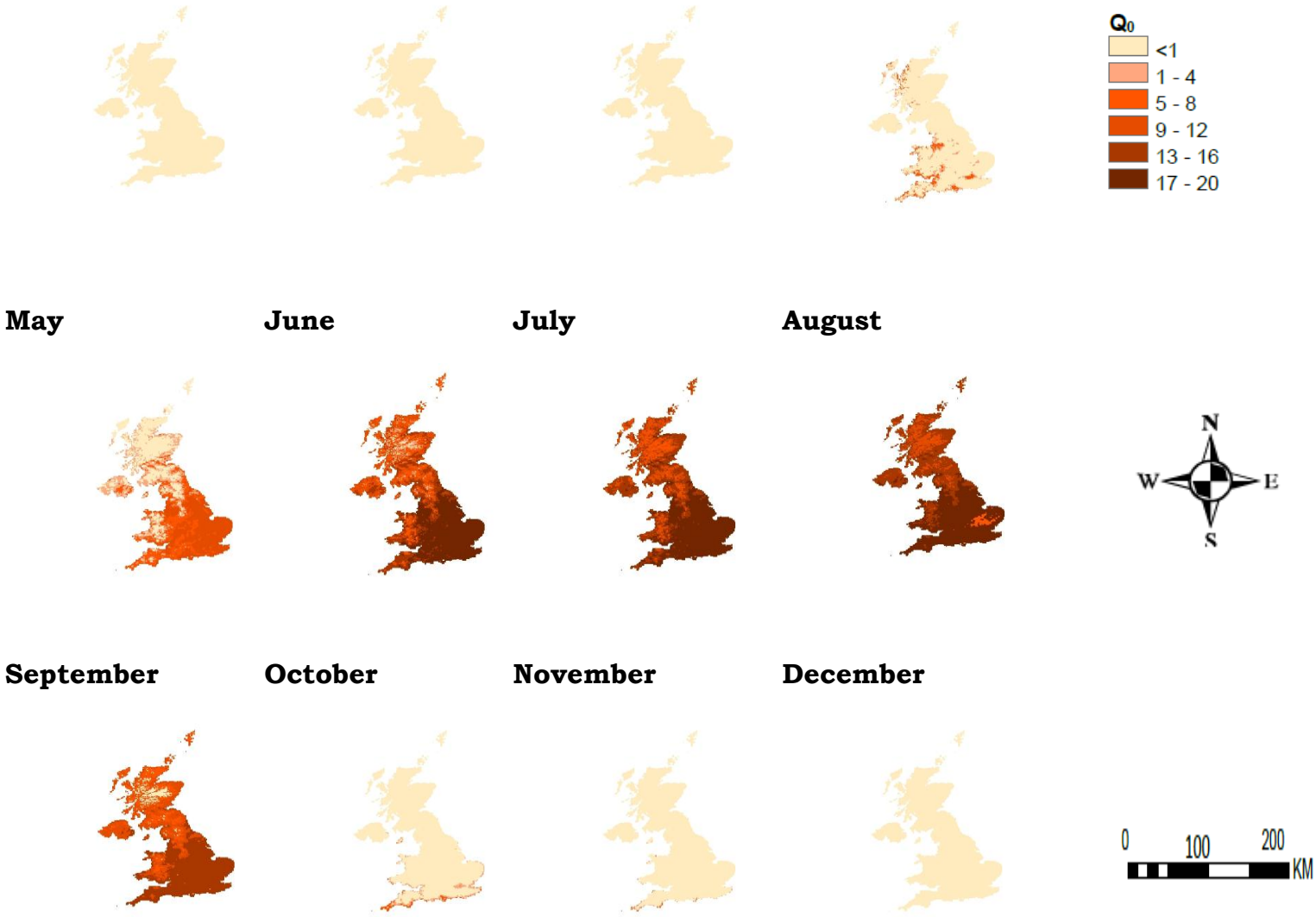


Figure 3: Average monthly Q0-based haemonchosis risk map showing the geographic distribution and relative risk of H. contortus infection pressure across the UK, 2001-2006

DISCUSSION AND CONCLUSION

This study shows that the Q_0 model is able to predict the timing of risks due to increased population of *H. contortus* or periodic changes in the infection pressures from the free-living stages of the parasite. By indicating the arrival of conditions suitable for the development and survival of infective larvae, high Q_0 values could trigger management measures such as treating sheep with anthelmintic to reduce egg output, or moving them to lower risk pastures. However, the model did not do so well in either location, when predicting variations in risk within the haemonchosis season. This deficiency is evident in the time-lag between predicted risk and the appearance of disease early in the season. Hypothetically, this is because the model cannot account for the following key biological processes associated with haemonchosis pathogenesis: hypobiosis, the time lag for larval development from eggs, the pre-patent period, accumulation of infection through time, physiological compensation for blood loss, and time lag in the decline of parasite numbers in the sheep population following the end of high risk periods for transmission (Kao *et al.,* 2000).

The cases in winter and/or early spring may in fact be accidental findings resulting from resumption of arrested fourth stage larvae or even burdens from the previous year. Q_0 is a model based on instantaneous parameters; however, in reality, egg development to *L3* and the pre-patent period are not instantaneous, but go through successive stages over a certain period. This could explain the early predictions of infection pressure before the actual incidence of disease. Furthermore, during unfavourable environmental conditions, *H. contortus* inhibits the maturation of the fourth stage larva, which is resumed once the climatic situations gradually become favourable (even though climatic conditions are not yet suitable for transmission outside the host). Therefore, the resumed maturation of hypobiotic larvae may be responsible for the cases when Q_0 predicted nil infection pressure from *L3* in winter and/or early spring (Fig.3). At that point in time; Q_0 indicates that, *H. contortus* infection pressure from the environment is absent because the prevailing climate is unsuitable for the availability of *L3* for infection. This is especially likely to be the case in late winter and early spring in the UK. However, during these periods, even though the environmental conditions are not conducive for availability of new free-living stages for transmission (as identified by Q_0), there can be very good survival of already developed *L3* at the temperature range of 7 – 9 °C. As such, some of the sheep population might still be suffering from parasite burden or are chronically infected, or indeed

newly infected by *L3* that persisted on pasture after the end of favourable conditions for development. In effect, these situations could be responsible for cases when infection pressure is predicted to be absent by Q_0.

Conclusively, it appears that the Q_0 model, based on its assumptions, is capable of transforming the prevailing climatic situations into the prediction of the periodic risk of haemonchosis transmission and occurrence. The model effectively predicts the periods of haemonchosis season, more importantly the peak point of each seasonal haemonchosis was predicted. Moreover, this study has been able to extend and establish the capability of the basic reproduction rate model as a monitor and predictor of the homogeneity or heterogeneity in the level of haemonchosis risk across different geo-climatic zones.

REFERENCES

Heesterbeek, J.A.P., Roberts, M.G., (1995). Mathematical models for micro parasites of wildlife. In GRENFELL, B.T., Dobson, A.P., eds, *Ecology of infectious disease in natural populations.* Cambridge: Cambridge University Press; pp. 90-122.

Kahn, L., (2010). A moisture index to predict development success of barber's pole worm and allow better management of pastures. *Turning the Worm* 26, 3-5.

Kao, R.R., Leathwick, D.M., Roberts, M.G., Sutherland, I.A., (2000). Nematode parasites of sheep: a survey of epidemiological parameters and their application in simple model. *Parasitol.* 121, 85-103.

Keeling, M.J., Rohani, P., (2008). *Modelling infectious diseases in humans and animals.* Princeton, N.J. and Oxford: Princeton University Press.

O'Connor, L.J., Walkden-Brown, S.W., Kahn, L.P., (2006). Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Vet. Parasitol.* 142, 1-15.

Roberts, M.G., Heesterbeck, J., (1995). Bluff your way into epidemiological models. *Trends Microbiol.* In press

Roberts, M.G., 1995. A pocket guide to host-parasite models. *Parasitology Today.* 11, 5, 172-177.

Rose, J.H., (1963). Observations on the free-living stages of the stomach worm Haemonchus contortus. *Parasitolgy*, 53: 469-481.

Scott, M.E., Smith, G., (1994). Parasitic and Infectious Diseases: Epidemiology and Ecology, *Academic Press.*

Silva, B.F., Amarante, M.R.V., Kadri, S.M., Carrijio-Mauad, J.R., Amarante, A.F.T., (2008). Vertical migration of Haemonchus contortus third stage larvae on Brachiaria decumbens grass. *Vet Parasitol.* 158, 85-92.

Smith, G., Grenfell, B.T., (1994). Modelling of parasites populations: gastrointestinal nematode models. *Vet Parasitol.* 54, 127-143.

Stromberg, B.E., (1997). Environmental factors influencing transmission. *Vet Parasitol.* 72, 247-264.

Tembely, S., Lahlou-kassi, A., Rege, J.E.O., Sovani, S., Diedhiou, M.L., Baker, R.L., (1997). The epidemiology of nematode infections in sheep in a cool tropical environment. *Vet Parasitol.* 70, 129-141.

EVALUATION OF *TEPHROSIA VOGELII* LEAF EXTRACTS FOR MOLLUSCIDAL PROPERTY ON LYMNAE

Tanko James Tenshak

Parasitology Division

Latankoj@yahoo.com

INTRODUCTION

Fasciola gigantica and *Fasciola hepatica* are parasitic trematodes that cause fasciolosis or liver rot disease of ruminants which is widely distributed in tropical, subtropical and temperate countries (Schillhorn Van veen, 1980). Lymnaeid snails are the main intermediate hosts (Soliman *et al.*, 2000). The incidence of snail-borne diseases is on the increase worldwide because of climate change and the development of land and water resources for agriculture. Livestock grazing in regions or areas that are frequently flooded or have a high water table are at high risk of becoming infected with liver fluke (Kanyari *et al.*, 2010). Snails act as amplifiers of the parasite by asexual reproduction, liberating large numbers of free-swimming cercariae that encyst on vegetation to infect animals and man (Alison, 2011). Control of the snail disrupts the life cycle of the parasite stopping the transmission of infection (Soulsby 1982). Several strategies have been employed to control snail population such as the synthetic organic molluscicides, but they are considered toxic to non target animals and may have long-term detrimental effects on the aquatic environment (Gehad *et al.*, 2009). In recent years, there has been increased attention on molluscicides that will be highly effective, rapidly biodegradable, and less expensive. Therefore, plant molluscicide could be appropriate for snail control measure against fasciolosis and schistosomiasis in endemic areas (Fayez, 2009).

In Nigeria, evaluation of many plants as potential molluscicides against different snail vectors have been carried out (Adewunmi and Sofowora, 1980; Kela, 1992; Azare *et al.*, 2002; 2007; Agboola *et al.*, 2012; Benson and Olajumoke 2012; Labe *et al.*, 2012 and Olofintoye, 2012). The aim of this study is to evaluate the molluscicidal properties of *T. vogelli* on lymnae snails.

Seminar presented 14th May, 2015 at NVRI Auditorium

MATERIALS AND METHODS

Snails/Plant materials

Fresh leaves of *Tephrosia vogelii* were collected in Vom, Jos South Local Government Area of Plateau State (Plate 1), identified and authenticated at the Herbarium, Department of Biological Sciences; Ahmadu Bello University Zaria, Nigeria. The leaves were air dried for two weeks at room temperature and pounded to a powdered form in a wooden mortar with pestle. Lymnae snails (Plate 2) were collected from the dam outlet of Ahmadu Bello University Zaria dam and Bassawa stream, in Sabo Gari Local Government Area of Kaduna state Nigeria.

A total of nine hundred and fifty two snails were collected into a sterile glass jar and transported to the Department of Parasitology and Entomology laboratory Ahmadu Bello University Zaria for identification and experimental studies. The snails were sorted and lymnaea snails identified and selected using the shape and size of the shells as described by Moema *et al.,* (2008).

The dried powdered leaf material weighing 250g were exhaustively and serially extracted with 1000ml of methanol and water respectively for 48 hours at room temperature of 27°C as described by Eman (2011). The extraction using each solvent was carried out three times and the extracts pooled together and kept in the desiccator. Each preparation was filtered separately through sterilized Whatmann No.1 filter paper. It was then concentrated in a rotary evaporator under vacuum to yield a semi-solid residue which was stored at 4° C until use (Agboola *et al.,* 2012).

Phytochemical Screening

Phytochemical analysis of the plant extract was conducted to determine the presence of the following; saponins, glycosides, alkaloids, flavonoids, tannins, steroid/terpenes and anthraquinones using standard procedures as described by Trease and Evans (1989) and Sofowora (1993).

Bioassay

The experimental design consists of positive control group exposed to standard molluscicide Niclosamide, negative control group exposed to 2% tween 80 in distilled water and positive test groups exposed to *Tephrosia vogelii* leaf extracted with water and methanol respectively. The bioassay was done according to WHO (1983) guide lines with slight modifications (Adenusi and

Odaibo, 2007; Farheen and Singh, 2012). The snails were exposed to the extracts at different concentrations of 50, 75, 100, 125, 150, 175 and 200 mg/l while a standard molluscicide, Niclosamide was used at concentration of 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, 17.5, 20.0 and 22.5mg/l. For each concentration, 10 medium size snails in three replicates were exposed and average mortality calculated after 6, 12, 18, 24 and 48 hours respectively. This was repeated for each of the different solvent extracts and the snails were observed at an interval of one hour for behavioural changes or mortalities. Mortality was checked using crushing technique as described by Adenusi and Odaibo (2008). At the end of the exposure time, test solution for each concentration was removed; snails were observed for sign of toxicity for a period of 24 hours and then washed five times using dam or stream water, kept in a new bowl for 24h post exposure and observed for resumption of activities as described by (Salawu and Odaibo 2011).

Probit value was plotted over the log concentration of the various extracts in mg/l and a correspondent line on the vertical and horizontal axis for the log of the concentration intercepted was taken as the LC_{50} and LC_{90} respectively (Kovendan *et al.* 2011).

Plate I: ***Tephrosia vogelii*** **plant.**

Plate 2: *Lymnaea* species used for the experiment

RESULT

Table 1: Results of phytochemical screening of *T. vogelii* leaf extracts

S/No	Test	Observation	Inference	Reference(s)
1.	Glycosides	Brown ring	+	Evans (1989) & Sofowora (1993)
2.	Alkaloid	Brown ppt	+	”
3.	Flavonoids	Yellow coloration	+	”
4.	Saponins	Persistent froth	+	”
5.	Anthraquinones	No colour change	–	”
6.	Steroids/terpenes	Reddishbrowncoloration	+	”
7.	Tannins	Blue-black	+	

Key: (+) indicates the presence of the compound tested for.

(−) indicates the absence of the compound tested for.

From the phytochemical screening the following compounds were present in the leaf extracts of *Tephrosia vogelii*; glycosides, alkaloid, flavonoids, saponins, steroids/ terpenes and tannins.
Tables 2-4 show the % mortalities of snails exposed to graded concentration of niclosamides and methanol extracts of *T. vogelii* respectively. Table 5 is the summary of the LC 50and LC 90 of the various preparations used in this study.

Table 2: Percentage mortality of snails exposed to niclosamide (Bayer®) a standard molluscicide at different concentrations (mg/l).

Conc.	Percentage (%)Mortality of snails over time (hours)				
(mg/l)	6	12	18	24	48
2.5	0	0	7	17	30
5	13	27	37	50	60
7.5	30	47	63	87	93
10	57	63	80	100	100
12.5	100	100	100	100	100
15	100	100	1000	100	100
17.5	100	100	100	100	100
20	87	100	100	100	100
22.5	100	100	100	100	100

Table 3: Percentage mortality of snails exposed to *Tephrosia vogelii* leaf water extract at different concentrations (mg/l).

Conc. (mg/l)	Percentage Mortality of snails over time (hours)				
	6	12	18	24	48
12.5	0	0	0	0	0
25	0	0	0	0	0
50	0	0	0	17	23
75	0	10	17	23	40
100	23	40	53	70	87
125	43	63	77	93	100
150	60	80	90	100	100
175	93	100	100	100	100
200	100	100	100	100	100

Table 4: Percentage mortality of snails exposed to *Tephrosia vogelii* leaf methanolic extract at different concentration (mg/l).

Conc.	Percentage (%)Mortality of snails over time (hours)				
(mg/l)	6	12	18	24	48
12.5	0	0	0	0	0
25	0	0	0	0	0
50	7	10	17	23	33
75	10	27	37	47	57
100	27	37	53	63	90
125	60	80	90	100	100
150	77	90	100	100	100
175	100	100	100	100	100
200	100	100	100	100	100

Table 5: Summary of the LC50 , LC90,of methanol and water extracts of *Tephrosia vogelii* and Niclosamide (Baylucide)

Solvent	LC50	LC90
Methanol	70.80 (mg/l)	154.80 (mg/l)
Water	100(mg/l)	177.83 (mg/l)

Niclosamide	5.01(mg/l)	7.94 (mg/l)

DISCUSSION

The molluscicidal properties observed were both time and concentration dependent using log-dose and time-log probit analysis for the determination of lethal concentration and exposure time (Teixeira *et al,* 2011). There was a significant difference ($P<0.05$) between concentration and mortality at 6, 12, 18, 24 and 48 hours of exposure to the extract, but at concentrations above 125mg/l, there was no significant difference ($P>0.05$) in mortality at the same exposure time between leaf extracted with methanol and water. Also as the concentration increased the exposure time required to cause mortality of the snail decreases. This agreed with Kloos *et al.,* (1987) that studied the molluscicidal activity of water extract of different parts of *T.vogelii* (leaves, stem and seeds). The behavioural changes of the snails observed in this study is characteristic for all molluscicides both plant and synthetic (Singh *et al.*, 1996; Salawu and Odaibo, 2011). Mortality observed might have occurred as a result of loss of water equilibrium between the snail and its habitat due to interference of the plant extracts. This may create anaerobic conditions that induce snail inactivity and its extrusion from the shell (Abdel-Aziz *et al.*, 1990; Osuala and Okwuosa,1993; Clark and Appleton, 1996; Brackenbury, 1999; Adetunji and Salawu, 2010). Other possible causes of mortality include, damage to the cutaneous respiration which therefore result in change in oxygen consumption.

CONCLUSION

Results from this study showed that the leaf extracts of *Tephrosia vogelii* has efficient molluscicidal activity against *Lymnaea* snails in a dose and time dependent gradient. *Tephrosia vogelii* may be a promising plant for the development of a cheap, safe and effective molluscicides in Nigeria.

REFERENCES

Abdel-Aziz, A., Brain, K. and Bashir, A.K. (1990). Screening of Sudanese plants for molluscicidal activity and identification of *Tacca leontopetaloides* (L) (Tuccaceae) as a potential new exploitable resource. *Journal of Phytotherapeutic Research,* 4: 62-65.

Adenusi, A.A. and Odaibo, A.B. (2007). Preliminary laboratory assessment of the crude aqueous and ethanolic extracts of *Dalbergia sissoo* plant parts for molluscicidal, ovicidal and cercaricidal activities.. *Travels Medicine Infectious Disease*. 6: 219-227.

Adenusi, A.A and Odaibo A.B. (2008). Preliminary laboratory assessment of the crude aqueous and ethanolic extracts of *Dalbergia sissoo* plant parts for molluscicidal, ovicidal and cercaricidal activities. *Travel Medicine Infectious Disease*. 6: 219-227.

Adetunji, V.O. and Salawu, O.T. (2010). Efficacy of ethanolic leaf extracts of Carica papaya and *Terminalia catappa* as molluscicides against the snail intermediate hosts of schistosomiasis. *Journal of Medicinal Plant Research,* 4(22): 2348-2352.

Adewumi, C.O. and Sofowora, E.A. (1980). Preliminary Screening of some Plant extracts for molluscicidal activity. *Planta Medica*, Journal of Medicinal Plants Research 39: 57-64

Agboola, O., Isaiah, A., Glory, O., Adesegun, S. and Adesanya S. A. (2012). Investigating the molluscicidal potential of some members of Nigeria sapindaceae family. *Archives of Applied Science Research*, 4 (3): 1240-1243.

Alison H. (2011). Snail-borne diseases in the bovids at high and low altitut in Eastern Uganda.Integrated Parasitological and Malaclogical mapping. An M.Sc Dissertation. Department of Biology, Control of Parasites and Disease Vectors.Liverpool School of Tropical Medicine.

Azare, B.A., Ebele, S.O. and Enwerem N. (2002). Relative molluscicidal potency of aqueous leaf extracts of *Alternanthera sesselis* on *Bulinus (Phy) globosus*. *Zuma Journal of Pure and Applied Science*, 1: 110-112.

Azare, B.A., Okwute, S.K and Kela, S.L (2007). Molluscicidal activity of crude water leaf extracts of *Alternanthera sesselis* on *Bulinus* (phy*) globosus. African Journal of Biotechnology,* 6(4): 441-444.

Benson, O. and Olajumoke A.M. (2012). Molluscicidal effect of aqueous and ethanolic extracts of Lemongrass (*Cymbopogon citratus*) leaf against the different developmental stages of *Biomphalaria Pfeifferi. New York Journal of Science,* 5(8): 70-76.

Brackenbury, T.D. (1999). Gross histopathological effects of an extract of *Agave attenuate* on the epithelium of the digestive tract of *Bulinus africanus. Annals of Tropical Medical Parasitology,* 93(5): 519-526.

Clark, T.E and Appleton, C.C. (1996). A semi quantitative approach to the selection of appropriate candidate plant molluscicides: A South African application. *Journal of Ethnopharmacology*, 56: 1-13.

Eman, E. Taher, Narmeen F. Mahmoud and Maha A. Mahmoud. (2011). LaboratorEvaluation of Some Egyptian Native Plants versus Some Parasitic Vectors. *Research Journal of Medicine and Medical Sciences,* 6(2): 85-90.

Farheen H. and Singh D. K. (2012). Molluscicidal activity of *Morus nigra* against the freshwater snail Lymnaea acuminata *Journal of Biology and Earth Sciences* 2: 14-23

Fayez, A. B. (2009). Use of some plant extracts to control *Biomphalaria alexandrina* snails with emphasis on some Biological effects. *World Applied Sciences Journal* 6(10): 1335-1345.

Gehad, T.E., Rawia, A.Z. and Eman, T.E. (2009).Molluscicidal activity of some *Solanum* species extract against the snail, *Biomphalaria alexandrina. Journal of Parasiitology Research,* 1-5 Doi. 10. 1155-47360.

Kanyari, P. W. N., Kagira, J. M. and Mhoma, J. R. L. (2010) 'Prevalence of endoparasites in cattle within urban and peri-urban areas of Lake

Victoria Basin, Kenya with special reference to zoonotic potential', *Scientia Parasitologica,* 11(4), 171-178.

Kela, S.L. (1992). Molluscicidal properties of *Polygonum limbatum* (L.). Paper presented at the 16th Annual Conference of the Nigerian Society for Parasitology,Ahamadu Bello University, Zaria,23-26th September,1992.

Kloos,H.,Thiongo,F.W.,Ouma,J.H. and Butterworth,A.E. (1987). Preliminary evaluation of some wild and cultivated plants for snail control in Machakos district, Kenya. *Journal of Tropical Medicine and Hygiene* 90: 197-204.

Kovendan, K., K. Murugan, C. Panneerselvam, P.M. Kumar, D. Amerasan, J. Subramaniam, S. and. Barnard, D.R.(2011). Laboratory and field evaluation of medicinal plant extracts against filarial vector, *Culex quinquefasciatus* Say (Diptera: *Culicidae*). *Parasitology Research*. 453-464.

Labe, Y., Inabo, H. I. and Yakubu, S.E. (2012). Comparative molluscicidal activity of aqueous and methanolic extract of *Zingiber offinale* against *Bulinus globusus*. *Advance in Enviromental Biology*, 6 (2): 831-835.

Moema, E.B.E., King, P.H., and Baker, C. (2008). Cercariae development in *Lymnae natalensis*. Krauss1848 collected in the vicinity of Pretoria, Gauteng Province South Africa. *Ondersteport Journal of Research,* 75: 215-223.

Olofintoye, L.K. (2012). Comparative evaluation of molluscicidal effects of *Seecuridaca longepedunculata* (Fres.) and *Tephrosia brateolate* (Guilland Perr) on *Bulinus globosus*. *New York Journal of Science* .5(8). Accessed August 28,2012*h t t pi/ www.science pub. Net/newyork.*

Osuala, F.O.U. and Okwuosa, V.N. (1993). Toxicity of *Azadirichta indica* to freshwater snails and fish with reference to Physicochemical. *In*: Kela

SL, Bowen ID (1995). Control of snail and snail – borne diseases. *Pesticide* outlook . 22 – 27.

Salawu, O. T. and Odaibo, A. B. (2011). The molluscicidal effects of *Hyptis suaveolens* on
different stages of *Bulinus globosus* in the laboratory. *African Journal of Biotechnology*, 10 (50): 10241-10247.

Schillhorn, van veen, T. W. (1980). Dynamics of *Lymnae natalensis* population in the Zaria area (Nigeria) and the relation to *Fasciola* gigantic infections. *Acta Tropica,* 37: 183-194.

Singh, A., Singh, D.K., Mishara, T.N. and Agarwal, R.A. (1996). Molluscicides of plant origin. *Biology Agriculture and Horticulture Journal,* 15: 205-252.

Sofowora, A. (1993). Recent trends in research into African Medicinal Plants. *Journal of Ethnopharmacology,* 38: 209-214.

Soliman, K.M, El-Ansary,A.K and Mohamed, A.M. (2000). Effect of carnosine administration on certain metabolic parametetres in bilharzial infected hamsters. *Journal of Egyptian Society of Parasitology.* 30: 455-468.

Soulsby, E. J. L. (1982). *Helminth, Arthropod and Protozoa of Domestic Animals.* 7th edition Baillere Tindall, London, UK.

Teixeira, T; Jose, S; Nuno, R; Baptista, J; Armindo, R (2011). Assessment of molluscicidal activity of essential oils from five Azorean plants against *Radix peregra* (Muller 1777), *Chemosphere* 87 (1-6), Elservier Ltd

Trease, G.E. and Evans, W.C. (1989). Text book of Pharmacognosy.Balliere Tindall: London (12): 176-180.

World Health Organisation (1983). Guidelines for evaluation of plant molluscicides. WHO. Report of scientific working group on plant molluscicide and guidelines for evaluation of plant molluscicide. Geneva , (TDR/SCH- SW (4)/ 83.3).

SOURCES OF *SALMONELLA* INFECTIONS IN SOME SELECTED POULTRY FARMS IN JOS, NORTHERN NIGERIA

Sati, N. M[1*]., Okolocha, E. C[2]., Kazeem, H. M[3]., Kabir, J[2], Fagbamila, I. O[4]., Muhammad, M[4] and Lettini, A. A[5].

[1] Poultry Division, National Veterinary Research Institute Vom, Nigeria.
[2] Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, Nigeria.
[3] Department of Veterinary Microbiology, Ahmadu Bello University Zaria, Nigeria.
[4] Bacterial Research Division, National Veterinary Research Institute Vom, Nigeria.
[5] OIE Italian Reference Laboratory for *Salmonella*, Istituto Zooprofilatico Sperimentale delle Venezie, Padova, Italy.

*__Correspondence__: snancykefas@yahoo.com. Tel: +2348026928771

INTRODUCTION

Poultry farming is an activity that is popular, both in rural and urban settings, in Nigeria. Poultry occupies a prominent position in the provision of animal protein and it accounts for about 25% of local meat production in Nigeria (Agbaje *et al.*, 2010).

Salmonella species are classified into serovars (serotypes) based on the lipopolysaccharide (O), flagellar protein (H), and sometimes the capsular (Vi) antigens. There are more than 2,500 known serovars (Popoff *et al.,* 2001; Chao *et al.,* 2007).

Small- scale poultry farmers in Nigeria lose up to 18% of chicks in the first two weeks of rearing. The mortality is often associated with salmonellosis and this exerts negative socio economic and food security impacts on farmers (Muhammad, 2008). The aim of this study is to identify sources of Salmonella infection on poultry farms in Jos, Plateau State.

Seminar presented 27th May, 2015 at NVRI Auditorium

MATERIALS AND METHODS

Sampling methods

Three (3) hatcheries and three (3) poultry farms in Jos, northern Nigeria were selected using a multistage random sampling method.

Evaluation of day old chicks (DOC) for *Salmonella* at the hatcheries

Eighteen samples per hatchery were collected from each of the three hatcheries. A total of 54 samples were collected at the hatcheries before the chicks were taken to their respective farms.

Evaluation of the chicks for *Salmonella* at the farms

Six samples each of faecal, feed, water and litter were collected from the nine farms every two weeks for 8weeks. Proper disinfection as well as change of laboratory wears was observed from farm to farm so as to check cross contamination.

Laboratory procedures

Faecal, feed, litter, water and tissue samples

The samples collected were processed using standard methods (Waltman *et al.*, 1998). One gram or litre (1g or 1l) of the sample was pre-enriched in buffered peptone water (BPW) in the ratio of 1:10 and incubated at 37^{o}C for 24 hours. Samples were then enriched on Rappaport Vassiliadis (RV) broth (0.1ml of sample from BPW into 10ml of RV broth) and incubated at 42^{o}C for 24 hours. Tissues from dead birds consisting of lungs, liver, spleen, caeca and heart were processed according to standard methods of isolation by Waltman *et al.* (1998). After enrichment, broth cultures were inoculated onto two selective media, Xylose Lysine Tergitol 4 (XLT4) and Brilliant Green Novobiocin Agar (BGN). Suspect colonies were then inoculated into Triple Sugar Iron (TSI) agar for 24hours at 37^{o}C. These isolates were sent to the *Salmonella* reference laboratory in Padova, Italy for serotyping following specific pattern of agglutination reactions using the Kauffmann-White classification scheme (Popoff *et al.*, 2001).

RESULTS

From the three hatcheries selected (hatchery A, B and C), two hatcheries (66.7%) had *Salmonella* isolated either from the tissues or faeces of birds before they were introduced to the different farms (Table1).

By the 14th day when the first farm visit was made, all the three farms from hatchery A maintained their *Salmonella*-free status. There was no trace of *Salmonella* in farm B3 where there was previous infection. Only farm C1 had S*almonella* and it was found in the feed, faeces and drinking water of the chicks. All the nine farms yielded no *Salmonella* on the three more farm visits except farm A3 where *Salmonella* was isolated during the third farm visit. Table 2 shows the distribution of *Salmonella* from the hatcheries to the farms during the course of this study

A total of 864 samples were taken after the chicks had been introduced to the nine farms and the total number of isolates obtained was 6 (0.7%). *Salmonella* Oakland, *Salmonella* Bonariensis *and Salmonella* Kentucky were obtained (Table 3). Eighteen tissue samples were analysed and four (22.2%) were positive for *Salmonella* (S. Oakland, *S. enterica subsp* Enterica). A total of 252 faecal samples were collected and 3 (1.2%) were positive for *Salmonella* (*S.* Oakland), while 2 (0.9%) out of the feed samples were positive (*S.* Oakland *and S.* Bonariensis). One water sample out of 216 (0.5%) (*S.* Bonariensis) and one (1) litter sample out of 216 (0.5%) were positive for *Salmonella* (*S.* Kentucky) (Tables 4 and 5).

Table 1: Table showing number of hatcheries tested for *Salmonella* in Jos, northern Nigeria

Hatchery	No of dead birds	No of dead birds positive	No of faecal samples	No of faecal samples positive	Total No of Samples	*Salmonella* Isolation (%)
A	6	Nil	12	Nil	18	Nil (0%)
B	6	1	12	Nil	18	1 (5.6%)
C	6	3	12	1	18	4 (22.2%)
Total	18	4	36	1	54	5 (27.8%)

Table 2: Distribution of *Salmonella* from the hatcheries to the farms during the course of this study

Farm visits	A1	A2	A3	B1	B2	B3	C1	C2	C3
Hatchery status	-	-	-	-	-	+	+	+	+

2weeks	-	-	-	-	-	-	+	-	-
4weeks	-	-	-	-	-	-	-	-	-
6weeks	-	-	+	-	-	-	-	-	-
8weeks	-	-	-	-	-	-	-	-	-

Key:
+ Positive for *Salmonella*
- Negative for *Salmonella*

Table 3: Serotypes of *Salmonella* isolated from the different farms sampled in Jos, northern Nigeria

Farm	Total no. of samples	No. *Salmonella* positive	Serotype isolated
A1	96	None	None
A2	96	None	None
A3	96	1	*S.* Kentucky
B1	96	None	None
B2	96	None	None
B3	96	None	None
C1	96	5	*S.* Oakland, *S.*Bonariensis
C2	96	None	None
C3	96	None	None
Total	864	6	

Table 4: Isolation of *Salmonella* from the different samples collected.

Type of sample	Number of samples	Number positive for *Salmonella* (%)
Tissues	18	4 (22.2)
Faeces	252	3 (1.2)
Feed	216	2 (0.9)
Water	216	1 (0.5)
Litter	216	1 (0.5)
Total	918	11 (1.2)

Table 5: *Salmonella* serotypes isolated from the different samples and their percentages

Isolate	Sample type (Number)	Number isolated (%)
S. Kentucky	Litter	1 (9.1)
S. enterica subspEnterica	Tissue	1 (9.1)
S. Oakland	Faeces(3),Feed (1)Tissues(3)	7 (63.6)
S. Bonariensis	Feed (1), Water (1)	2 (18.2)

DISCUSSION

Epidemiological studies have demonstrated a variety of routes through which *Salmonella* can be disseminated within a poultry enterprise (Nayak *et al.,* 2004). *Salmonella* may infect young chicks directly through ovarian transmission or penetrate the egg shell after the egg has been laid (Cox *et al.*, 2000). Newly hatched chicks are at their peak of susceptibility to *Salmonella* (Gast, 2007). Poultry can become infected by horizontal transmission through infected litter, faeces, feed, water, dust, fluff insects, equipment, fomites, contaminated with *Salmonella* (Poppe, 2000), and some of these have been seen in the course of this study. These findings are in agreement with the report of Muhammad *et al.* (2010), who isolated *Salmonella* from day old chicks from hatcheries in Jos, Nigeria.

Environmental sources are some of the ways *Salmonella* gets into poultry farms. Numerous environmental factors can influence the likelihood and outcome of infections of poultry with *Salmonella*. Lengthy environmental persistence of pathogens can generate extended opportunities for horizontal transmission within and between flocks (Gast, 2007). In this study, *Salmonella* from environmental sources account for 63.6% (7 out of 11) of the isolates obtained. Isolating the organism from the environment is difficult because of the few salmonellae in these sources (Waltman *et al.*, 1998) and the fragility of the organism in these samples.

It has been reported that bacteriological sampling does not always provide an accurate indication of infection within a flock because of low incidence of infection and the intermittent excretion of *Salmonella* organisms (Hassan *et al.,*

1990). This might also explain why the rate of isolation of the organism was low in this study.

CONCLUSION AND RECOMMENDATIONS

Salmonella infections are not just acquired on farms, but sometimes the hatcheries that supply chicks are responsible for disseminating the organisms as was seen in this study, resulting in high chick mortality, poor feed conversion and unnecessary exposure of farmers/consumers to infections.
Government should enforce the existing laws that prohibit establishment of poultry farms and hatcheries without adequate training for farmers.
Government should provide veterinary and animal health extension services in all the local government areas to ensure adequate records, proper monitoring, and effective management of *Salmonella* infections.
Surveillance is needed to help prevent food-borne disease outbreaks and raise awareness among health authorities, food producers, food regulators, and consumers.

REFERENCES

Agbaje, M., Davies, R., Oyekunle, M.A., Ojo, O.E., Fasina, F.O. and Akinduti, P.A. (2010).Observation on the occurrence and transmission of *Salmonella gallinarum* in commercial poultry farms in Ogun State, South Western Nigeria. *African Journal of Microbiology Research* 4(9), 796-800.

Chao, M.R., Hsien, C.H., Yeh, C.M., Chou, S.J., Chu, C., Su, C.Y. and Yu, C.Y. (2007). Assessing the Prevalence of *Salmonella enterica* in Poultry Hatcheries by Using Hatched Eggshell Membranes. *Poultry Science* 86:1651-1655.

Cox, N.A., Berrang, M.E., Cason, J.E. (2000). *Salmonella* Penetration of egg shells and proliferation in broiler hatching eggs: a review. *Poultry Science* 79:1571–1574.

Gast, R.K. (2007).Serotype-specific and serotype-independent strategies for preharvest control of food borne *Salmonella* in Poultry. *Avian Diseases* 51:817-828.

Hassan JO, Barrow PA, Mockett APA, McLeod S (1990). Antibody response to experimental *Salmonella typhimurium* infection in chickens measured by ELISA. *Veterinary Records*. 126: 519-522.

Muhammad, M (2008). The role of *Salmonella sp* in early chick mortality and the application of hazard analysis critical control point (HACCP) for control. Ph.D Thesis University of Maiduguri

Muhammad, M., Muhammad, L.U., Ambali, A., Mani, A.U., Azard, S. and Barco, L. (2010) Prevalence of *Salmonella* associated with chick mortality at hatching and their susceptibility to antimicrobial agents, *Veterinary Microbiology* 140: 131-135.

Nayak, R., Stewart, T., Wand, R.F., Lin, J., Cerniglia, C.E., Kenney, P.B (2004) Genetic diversity and virulence gene determinants of antibiotic resistant *Salmonella* isolated from preharvest turkey production sources. *International Journal of Food Microbiology* 91: 51–62.

Popoff, M.Y., Bockemühl, J., Brenner, F.W.and Gheesling, L.L. (2001). Supplement 2000 (no. 44) to the Kauffmann-White scheme*, Research in Microbiology, 152:907–909*.

Poppe, C. (2000). *Salmonella* infections in the domestic fowl, in Wray, C. & Wray, A. (Eds) *Salmonella* in domestic animals, (Wallingford CABI Publishing). Pp 107-132.

Waltman, W.D., Gast R.K. and Mallinson E.T. (1998*).* Salmonellosis; A laboratory manual for the isolation and identification of avian pathogens In: Swayne D.E., Glisson J.R., Jackwood M.W., Pearson J.E and Reed W.M (Eds) *American Association of avian pathologists 4th edition*. University of Pennsylvania, USA, 4-13.

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *SALMONELLA* SEROVARS FROM CATTLE IN JOS, NIGERIA

Mercy N. Benson
Quality Control Division
National Veterinary Research Institute, Vom
E mail: mercydidel@yahoo.com

INTRODUCTION

Salmonellae are zoonotic bacteria with a rising prevalence in the cattle industry (Randall, 2001). Salmonellosis remains a global problem with a significant economic impact on the cattle industry causing livestock mortality, abortion and reduced milk production. They are among the most common bacterial food-borne pathogens worldwide (Todd, 1997). Under-reporting of salmonellosis and the presence of other diseases considered to be of higher priority may have over-shadowed the problem of the disease in some developing countries. In Nigeria, efforts at prevention and control have largely been by improved conditions on grazing reserves, improved sanitation and hygiene (Adene and Oguntade, 2008), improvement in public water supply, safe disposal of waste, use of newer antimicrobials and general public health measures (Adeniran *et al.*, 2005).

This study was designed to determine the serovars and antimicrobial susceptibility profile of *Salmonella* species from cattle in Jos, Nigeria.

MATERIALS AND METHODS

Sample collection

A total of 712 faecal samples were collected from cattle from 3 sites including cattle farms (572), cattle control-post (60) and an abattoir (80). The 712 cattle comprised of 164 males and 548 females, 120 calves and 592 adults, 609 White Fulani cattle, 3 Cross breeds and 100 Holstein Friesians. One hundred were raised under intensive system, 224 under semi intensive, 248 under extensive while 140 were unknown (control post and abattoir). Faecal samples were obtained per rectum and packed in a cold box and then transported immediately from point of collection to the laboratory for examination.

Isolation and serotyping

Salmonellae were isolated and identified according to the techniques recommended by the International Organization for Standardization (ISO) 6579

(2002) from pre-enrichment to serotyping (which was done in The *Salmonella* Reference Laboratory Padova, Italy).

Antimicrobial susceptibility profile

The antimicrobial susceptibility pattern of the isolates was conducted using a modified Kirby-Bauer disk diffusion method (Bauer *et al.,* 1966). A panel of 16 antibiotics namely: colistin (10 µg), sulphamethoxazole + trimethoprim (23.75 µg +1.75 µg), kanamycin (30 µg), gentamicin (10 µg), cefotaxime (30 µg), amoxicillin + clavulanic acid (20 µg + 10 µg), ceftazidine (30 µg), nalidixic acid (30 µg), tetracycline (30 µg), ampicillin (10 µg), streptomycin (10 µg), triple sulfa (0.25 µg), chloramphenicol (30 µg), cephalothin (30 µg), enrofloxacin (5 µg), ciprofloxacin (5µg) were tested. Each isolate was diluted in sterile saline solution to a 0.5 McFarland standard. The diluted bacterial suspension was transferred onto Mueller Hinton agar plates using sterile swabs. The plates were seeded uniformly by rubbing the swabs against the entire agar surface. Each antimicrobial impregnated disk was applied onto the surface of the inoculated plate by using a sterile disc dispenser. The plates were incubated at 37^0C for 18 hours. Interpretation of the growth inhibition zones and classification of isolates as susceptible, intermediate and resistant was done following guidelines of the National Clinical Laboratory Standards (NCCLS, 2004).

Statistical analysis

The occurrence of *Salmonella* was calculated using percentages and the results were analysed to determine if any relationship existed between the presence of *Salmonella* and age, sex, breed and management system using Chi square test by Statistical Package for Social Sciences (SPSS) Version 15 (Inc.,Chicago,USA).

RESULTS

An isolation rate of 2.95% (21 of 712) was recorded in this study. Higher isolation rates were recorded in adults (3.21%), females (4.19%),White Fulani breeds (3.28%) and extensively managed cattle (6.45%) compared to that in calves (1.67%), males (1.22%), Holstein Freisian (1%) and Cross-breeds (0%), intensively (1%) and semi-intensively bred cattle (1.34%) respectively. Isolation rates of 6.29%, 3.06% and 1.59% were recorded in Jos-East, Jos-North and Jos-South respectively. Four serotypes were isolated; *S.* Pullorum (43.48%), *S.* Hull (34.8%), *S.* Poona (13.04%) and *S.* Rubislaw (8.7%) (Table 2). The

occurrence rate of *S.* Pullorum in Jos-East, Jos-South and Jos-North L.G.As were 54.5%, 36.4% and 9.1% respectively. In addition, there were mixed infections in some samples resulting in 23 isolates. Most of the isolates (91.6%) were susceptible to all the antimicrobials tested except colistin, streptomycin and triple sulfa (9.1%) to which some isolates were resistant. There was a significant association (P<0.0012) between the presence of *Salmonella* and site. There was also a significant association between the presence of *Salmonella* and management system (P=0.005). However there was no significant association (P>0.05) between the presence of *Salmonella* and age, sex, breed and site.

Table 1: **Frequency of isolation of *Salmonella* based on source of samples**

Site	No collected	No positive (%)
Cattle farms	572	19 (3.32)
Abattoir	80	2 (2.5)
Control post	60	0 (0)
Total	712	21 (2.95)

P = 0.3401

Table 2: Distribution of *Salmonella* serotypes isolated from the three sampling sites

Serotypes	Farms	Abattoir	Control-post	Total (%)
S. Pullorum	9	1	0	10 (43.48)
S. Poona	2	1	0	3 (13.04)
S. Rubislaw	2	_	0	2 (8.70)
S. Hull	7	1	0	8 (34.8)
Total	12	3	0	23 (100.02)

DISCUSSION

*Salmonella*e were isolated from cattle on farms and those slaughtered for meat at the abattoir. Overall, 2.95% (21 of 712) of cattle sampled were positive for *Salmonella* species. The low isolation rate of *Salmonella* obtained in this study might be due to single sampling per animal, season, regional variation, management practices or a low carrier rate in cattle in the study area at the period of sampling.

In this study, *Salmonella* was isolated in all the three localities sampled though Jos-East had a higher isolation rate with a statistically significant value ($P<0.05$) compared to Jos-North and Jos-South. The higher isolation rate in Jos-East could be attributed to the movement of high population of cattle (especially nomadic) through the area.

The higher frequency of isolation on farms, though not statistically significant ($P>0.05$), compared to the abattoir or control-post was likely due to the lowered gastric acidity in the animals from starvation during movement from the farms to the abattoir.

The extensively managed cattle produced the highest isolation rate with a statistically significant value ($P < 0.05$) compared to those on semi-intensive or intensive farms. This was attributed to their mode of feeding which is majorly random grazing where they may be exposed to various pathogens.

Interestingly, from the 21 positive samples, host adapted *Salmonella* (*S.* Pullorum) had the highest occurrence rate compared to the other serotypes. This observation may be associated with random grazing habits of the cattle in different regions including areas where poultry farms are situated or where poultry have roamed, thereby contaminating the pasture with poultry faeces/wastes. In addition, potential sources of infection arise when poultry waste is released into waterways and is thereafter used to irrigate livestock forage crops or inadvertently contaminates pasture on which cattle graze or is ingested via drinking.

Other serotypes: S. Hull, S. Poona and S. Rubislaw were isolated in a decreasing order respectively. These serotypes or other environmental *Salmonella* may be the cause of food-borne outbreaks, in particular from fruits, vegetables and spices contaminated by *Salmonella* from feral reptiles or other animals. This may explain recent outbreaks of *S.* Poona in the USA (Molbak *et al.,* 2012). Reports of S. Poona and S. Rubislaw have been made in Nigeria by Olayemi (1978) in sewage waste from the abattoir in Zaria and Collard and Sen (1956) in Ibadan respectively. Stevens *et al.* (2006) equally isolated *S.* Poona from beef in Senegal. If more colonies per sample had been

picked, we might have found more serotypes, masked by dominant ones. In this work however, isolated colonies were randomly selected among characteristic ones for confirmation. The bias is therefore weak, but a different competitiveness for each serotype cannot be excluded. The serotypes obtained in this study may reflect the serotypes prevalent in this environment.

Majority of the isolates were susceptible to the panel of antimicrobials. However, 2 isolates were resistant to sulfonamide and 1 isolate each were resistant to colistin and streptomycin. Among the 23 serotypes, 8.69% displayed resistance to at least one antimicrobial. In contrast 91.3% were sensitive to all the antimicrobials. Development of resistance to sulfonamides may be as a result of indiscriminate use of the antimicrobial in animal husbandry in the area. In Ethiopia, Alemayehu *et al.* (2003) reported that 52% of the *Salmonella* isolated at the slaughterhouse from beef were resistant to at least three antimicrobials. In the United States, 84% of the *Salmonella* isolates from retail meats were resistant to at least one antimicrobial, and 53% to at least three antimicrobials (Duffy *et al.*, 1999). By comparison therefore, the strains isolated from Jos showed a low level of resistance to commonly used antimicrobials. Among the serotypes, only *S.* Poona manifested multiple drug resistance. This resistance was exhibited to sulphonamide, which may be due to inappropriate use of antimicrobials in the area due to quackery.

CONCLUSIONS

A 1ow overall *Salmonella* occurrence rate of 2.95% in cattle in Jos-Nigeria was recorded in this study. Samples from the control-post in Jos failed to yield *Salmonella* isolates. The data documented rare or unusual serovars of *Salmonella* in cattle (*S.* Poona*, S.* Rubislaw*, S.* Hull and *S.* Pullorum). To the best of our knowledge, this is the first report of such *Salmonella* serotypes in cattle in the study area. This result is significant because *S.* Pullorum is known to be a poultry associated serotype and coincidentally recorded the highest occurrence among the serotypes found, implying that the serotype may have a wider host range.

RECOMMENDATIONS

We recommend that there is need to improve surveillance especially at control-posts to avoid spread and introduction of new serotypes into clean areas. Information on *Salmonella* isolates, their antimicrobial susceptibility patterns and virulence characteristics need to be passed on to medical practitioners. Public education on the need for hygienic slaughter, handling, processing or cooking of meat should be enhanced.

REFERENCES

Adene, D.F. and Oguntade, A. E. (2008). *Poultry sector country review*: Nigeria, pp 15

Adeniran, J.O, Taiwo, J.O. and Abdu-Rahaman, L.O (2005). *Salmonella* intestinal perforation; 27 perforations in one patient, 14 perforations in another. Are the goal post changing. *Journal of Indian Association of Pediatric Surgery*, 10(4): 248-252.

Alemayehu, D., Molla, B., Muckle, A. (2003). Prevalence and antimicrobial resistance pattern of *Salmonella* isolates from apparently healthy slaughtered cattle in Ethiopia. *Tropical Animal Health Production,* 35: 309–319.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American *Journal of Clinical Pathology*, 45:493-496.

Collard, P and Sen, R. (1956). Isolation of *Salmonellae* from cattle in Ibadan. *West African Journal of Medicine*, 5: 118-120.

Duffy, G., Cloak, O.M., O'Sullivan, M.G., Guillet, A., Sheridan, J.J., Blair, I.S., McDowell, D.A. (1999). The incidence and antibiotic resistance profiles of *Salmonella spp.* on Irish retail meat products. *Food Microbiol*ogy, 16: 623–631.

ISO. (2002) International Organization for Standardization 6579. Microbiology of food and animal feeding stuff-Horizontal method for the detection of *Salmonella*, Geneva. PP. 1-18.

Mølbak, K., Olsen, J. E. and Wegener, H.C (2012) *Salmonella* infections, retrieved on July 12, 2012.12.35 am from www.123foodscience.com/food_microbiology/*Salmonella*_ infections.

NCCLS (2004) National Clinical Laboratory Standards, Performance standards for antimicrobial disk susceptibility testing. NCCLS document M100-514.Wayne, Pa.

Olayemi, A. B. (1978). The incidence of *Salmonella* in cattle slaughtered at the Zaria abattoir, M. Sc. Thesis, Ahmadu Bello University, Zaria, Nigeria.

Randall, J.B. (2001). *Salmonellosis in cattle* 1:8 Retrieved on 17 April, 2010, from www.the cattle site.com/*Salmonella*-dublin infection.

Stevens, A., Kaboré, Y., Perrier-Gros-Claude J., Millemann,Y., Brisabois, A., Catteau, M., Cavin, J. and Dufour, B. (2006). Prevalence and antibiotic-resistance of *Salmonella* isolated from beef sampled from the slaughterhouse and from retailers in Dakar (Senegal). *International Journal of Food Microbiology,* 110: 178–186.

Todd, E.C.D (1997). Epidemiology of food-borne diseases: A worldwide review. *World Health Statistics Quarterly*, 50: 30–50.

REVERSE GENETICS: A USEFUL TOOL IN FUNCTIONAL GENOMICS

(ROLE OF *ZNT1* GENE IN VERTEBRATE DEVELOPMENT)

Muraina Issa[1,2], Graham Anthony[3] and Hogstrand Christer[2]

[1] National Veterinary Research Institute, PMB 01, Vom, Nigeria
[2]King's College London, Faculty of Life Sciences and Medicine, Diabetes and Nutritional Sciences Division, Metal Metabolism Group, Waterloo Campus, UK
[3]King's College London, MRC Centre for Neurodevelopmental Biology, Guy's Campus, UK

Correspondence*:* *muravet2002@yahoo.com*

INTRODUCTION

Reverse genetics methodologies generally refer to the generation or targeted discovery of a mutation in a gene that is known by its sequence. It is a useful approach to discover gene's function by analysing the phenotypic effect of specific gene sequence. This is a reverse of the so-called forward or classical genetics in which the investigative process proceeds in opposite direction. That is, while forward genetics attempts to find the genetic basis of a phenotype or trait, reverse genetics aims to find what phenotype arises as a result of particular genetic sequences. Different effective reverse genetics approaches are in existence but they tend to be organism specific. Examples include gene knockout via homologous recombination in mice (and recently in *Drosophila*), gene knock-in via insertion (transgene) in mouse and gene knockdown or silencing via antisense morpholino oligonucleotides in *Xenopus* and zebrafish oocytes or via RNA interference (RNAi) in *Ceanorhabditis elegans, Drosophila* and mouse. Other methods include TE (Transposable Elements) in *Drosophila* and *Ceanorhabditis elegans*, and TILLING (Targeting Induced Local Lesions in Genomes) which is applicable to most organisms identifies mutations in specific genes of interest in chemically mutagenized populations (Stemple, 2004).

Among the mammalian zinc transporter genes, *ZnT1* (*SLC30A1*) was the first to be discovered with its expression throughout the body notably in the basolateral membranes of epithelia involved in zinc acquisition or recycling like intestine, kidney and placenta (Lichten and Cousins, 2009) and functions to transport excess cytoplasmic zinc out of the cells (Palmiter and Findley, 1995).

Previous works have shown that homozygous deletion or knockout of the whole *ZnT1* gene in mice was embryonic lethal (Andrews *et al.*, 2004) whereas in *Caenorhabditis elegans* as well as in *Drosophila*, a loss-of-function mutation of their respective ZnT1 orthologs result in animal with impair growth and development (Bruinsma *et al.*, 2002; Wang *et al.*, 2009).

The zebrafish (*Danio rerio*) is an important vertebrate model system; well suited for studies in genetics, embryology, development, and cell biology because of the unique characteristics it possesses (Westerfield, 1993). In addition, there is strong conservation between zebrafish and humans for most genes, which makes zebrafish an exellent model organism for studying complex biological processes (Chen and Fishman, 1996). With the advent of TILLING technology, it is now possible to generate zebrafish knock-outs or mutants for specific genes, making reverse genetics available for this model.

METHODOLOGY

Animal model

Zebrafish embryos (strains sa0017) recovered from the out-crossing of a wild-type female fish and a male fish that is heterozygous for *slc30a1* (*znt1*) gene mutation (using TILLING technology) were collected from Sanger Centre Zebrafish Mutation Project (http://www.sanger.ac.uk/cgi-bin/Projects/D_rerio/zmp/search.pl?q=slc30a1). This mutation caused a transition of nucleotide "A" to "T" at 355 nucleotide sequence leading to premature termination of the Znt1 protein sequences which resulted in a truncated protein, short of the last forty (40) amino acids. These embryos were reared to adult in a stand-alone zebrafish facility of King's College London under standard fish husbandry practice and thereafter different genotypes were classified. Fish were bred and embryos from different genotypes were collected for study.

In a related experiment, wild-type embryos were micro-injected at 1-4 cell stage with 2-4ng of translational blocking anti-sense morpholino-modified oligonucleotides (MO) for *znt1* gene knockdown (5'GCGGAGCACAGACAGAAACAAAAGCT3') (GENETOOLS, Philomath, USA) as previously described (Nasevicius and Ekker, 2000). A scramble or mismatch MO (Random-control-MASO) was injected in a similar way to serve as injected control along with un-injected wild-type control. Because of the potential problem of off-target effects produced by most MOs as a result of *p53* gene activation causing a non-specific neuronal cell death (Pickart *et al.,* 2005), a *p53* translational blocking MO (5'GCGCCATTGCTTTGCAAGAATTG3') was co-

injected with *znt1* MOs in the ratio of 1.0 : 1.5 to suppress the *p53*-mediated apoptosis, and the result of the embryonic development was compared to that resulting from injecting *znt1* morpholino alone. All embryos were incubated at 28.5^{o}C and monitored through developmental stages.

For exposure analysis, embryos were incubated in embryo water either supplemented with zinc by 100µM of $ZnSO_4$ or depleted of zinc by 5µM TPEN (N,N,N,N,-Tetrakis(2-pyridymethyl ethylene-diamine).

Mutation detection and genotype classification

A simple and rapid Locked Nucleic Acid (LNA) method was adapted for detection of SNP (single nucleotide polymorphism) mutation which was used to classify the genotype (Johnson *et al.,* 2004). This is based on TaqMan assay which utilizes dual-labelled fluorescence probes to discriminate between allele A and T in the target region of the genomic DNA of fish using a Real-Time PCR (qPCR) technique. The primers and probe sequence sets for the assay were designed and synthesized by Integrated DNA Technology (IDT). This simple and cost-effective method of mutation detection was re-confirmed in a number of samples using conventional nested PCR followed by sequence analysis of the target region. The primers for conventional PCR were designed by Sanger Centre's Zebrafish Mutation Project and synthesized by Sigma. In this technique, both internal forward and reverse primers were further re-designed with M13 forward and reverse tail primers for bi-directional sequencing of the PCR products.

Gene expression analysis

DIG-labelled anti-sense RNA for *znt1* gene (probe) was produced according to standard gene cloning procedures followed by *in vitro* transcription. The forward and reverse primer sequences used for amplification of the gene fragment gave a product size of 538bp (f: 5'AGACCCAGTCCACCAACAAG3'; r: 5'AGGACATGCAGGAAAACACC3'). This probe was used for gene expression analysis on 24hpf embryos using the method of whole mount *In Situ* Hybridization (ISH) as described by Thiese *et al.,* 2004.

Total and free zinc analysis in embryo

Chorionated embryos at 24hpf of *znt1* homozygotes, *znt1* morphants and wild-types backgrounds were acid digested respectively and total zinc concentrations were measured using ICP-MS (Perkin Elmer, model ELAN 6100DRC) as previously described (Zheng *et al.,* 2008). In a related experiment, free zinc (Zn^{2+}) ions were assayed in the embryos by both fluorescent spectrophotometer and fluorescent microscope at 360/530nm excitation/emission wavelength using a synthetic ratiometric zinc-specific

fluorophore termed “ZTRS” probe which was a kind gift from Dr. Zhaochao Xu of the University of Cambridge.

Phosphorylated- extracellular regulated kinase (ERK) 1/2 activity in embryos

Embryos at early to mid gastrulation stages (5-8hpf) were used for phospho-ERK immuno-staining technique for Znt1 homozygote mutants, Znt1 morphants, control wild-type and control morphant. A primary antibody to phospho-ERK1/2 (Cell Signalling®; rabbit polyclonal, #4370S) was used at 1:100 dilutions and a secondary antibody (Abcam®; goat anti-rabbit conjugated to Alexa Fluor 488, ab15007) was used at 1:200 dilutions as previously described (Krens *et al.*, 2008). Expression of p-ERK1/2 proteins in stained embryos were observed under epi-fluorescence microscope. In a parallel experiment, about 10µg of protein was extracted from 24 hpf embryos of wild-types and homozygote mutants respectively and analysed for phospho-ERK by Western blotting as previously described (Krens *et al.*, 2008), using rabbit polyclonal p-ERK1/2 as the primary antibody at 1:2,000 and goat anti-rabbit conjugated to horse radish peroxidase (GE Healthcare Life Sciences, RPN2108) as the secondary antibodies at 1:5,000. Antibodies to total ERK 1/2 (Cell Signalling®; #9102S) were used as normaliser or reference protein at 1:1000 along with the secondary antibody at 1:2,000. Films (blots) were processed and developed by automatic machine (Konica Minolta SRX-101A).

RESULT AND DISCUSSION

Bioinformatics

The result of bioinformatics analysis using ClustalW Multiple Sequence Alignment of orthologue Znt1 proteins between teleosts and mammals revealed a high degree of homology between zebrafish and mammals including human (i.e. 96% similarity and 56% identity with human), suggesting that this protein perform similar function in the species (data not shown). Analysis by Eukaryotic Linear Motif (ELM) search of truncated (40 amino acids) region in the C-termini of Znt1 revealed some evolutionarily conserved residues between human ZnT1 and zebrafish Znt1 especially the PDZ domain containing the “ESSL” motif, suggesting that the truncation of the C-terminus in zebrafish Znt1 might affect the function of the protein (Fig 1).

Znt1 deficiency or disruption caused delay in stages of embryo development

The result of growth and development in zebrafish (Fig 2A & 2B) showed that embryo with Znt1 homozygote mutation was viable unlike mouse embryo with ZnT1 null mutation which was embryonic lethal at 9th day of gestation (Andrew *et al.*, 2004). This may be as a result of partial loss-of-function or altered function in the zebrafish mutation compared to the complete loss-of-function in

the ZnT1 knockout mouse. The disturbances or delay in the stages of growth and development observed in zebrafish embryos with Znt1 deficiencies coupled with the observed growth acceleration by zinc removal with TPEN or further growth retardation by zinc supplementation (Fig 2A) suggested that Znt1-deficient embryos have increased zinc accumulation probably due to defect in Znt1 protein trafficking zinc out of the cytosol.

Znt1 disruption reduced expression of *znt1* gene

The reduced expression of the *znt1* gene in the Znt1 mutant and the Znt1 morphant embryos especially around the yolk syncytial layer (YSL) and the central nervous system (CNS) as observed in the present study by ISH (Fig. 2C) may point towards the role of Znt1 in these regions (Thisse *et al.*, 2004). This may explain the behavioural disturbances (circling) or reduced muscle mass sometimes observed in morphants (Fig 2B) probably as a result of the defective gene causing reduced expression of the protein normally required to regulate zinc in those tissues and also around the YSL, which may be required for zinc uptake from the yolk to the developing embryo. This may possibly result in a mutant or morphant embryo with reduced ability to absorb zinc/nutrient from the yolk, resulting in reduced muscle mass and enlarged yolk (red and black arrows in Fig. 2B respectively) with increased zinc accumulation. This thus lessen zinc availability for fish development as previously demonstrated in a rodent model with defective zinc flux from placenta or yolk sac membrane to the growing embryo (Andrews *et al.*, 2004).

Znt1 deficiencies caused increased free Zn^{2+} accumulation in embryos

Total zinc concentration by ICP-MS showed no statistical significant difference between the genotypes but there is a tendency of homozygote mutant to accumulate higher zinc level than the wild-types and this was confirmed by using "ZTRS" zinc fluorescent probe to measure free Zn^{2+} level in chorionated embryos (Fig 3A & B). The increased fluorescence Zn^{2+} signal with concomitant impaired embryonic development observed in Znt1 homozygote and morphant zebrafish embryos was supported by previous work with *Drosophila* Znt1 mutant (Wang *et al.*, 2009).

Znt1 deficiencies caused reduced phospho-ERK 1/2 signalling in embryos

There was reduction in the activation or signalling of phospho-ERK1/2 in Znt1 homozygote mutant and Znt1 morphant embryos compared to wild-type embryo (Fig. 4A) and this might be responsible for the delay development observed in mutant embryo which was supported by previous experiment with *C. elegans* having a loss-of-function mutation of the *cdf-1* gene (an homologue of zebrafish *znt1*) (Bruinsma *et al.*, 2002). This defect may be connected to an impaired Ras-ERK (MAPK) signalling pathway in mutant worms (Bruinsma *et*

al., 2002) or in *Xenopus* oocytes, which also showed a delayed meiotic maturation when the gene was disrupted and expressed in frog oocytes (Jirakulaporn and Muslin, 2004). These observations lend further credence to support the interaction between ZnT1 and MAPK pathway activation being necessary for diverse cellular process including cell growth, proliferation, differentiation, survival and vertebrate development (Ballif and Blenis, 2001).

REFERENCES

Andrews GK, Wang H, Dey SK, Palmiter RD (2004). Mouse zinc transporter 1 gene provides an essential function during early embryonic development. *Genesis* 40: 74-81.

Ballif BA, and Blenis J (2001). Molecular mechanisms mediating mammalian mitogenactivated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. *Cell Growth Diff.*, 12:397-408.

Bruinsma JJ, Jirakulaporn T, Muslin AJ, Kornfeld K (2002). Zinc ions and cation diffusion facilitator proteins regulate Ras-mediated signaling. *Dev. Cell.* 2: 567-578.

Chen JN, Fishman MC (1996). Zebrafish tinman homolog demarcates the heart field and initiates myocardial differentiation. *Development* 123:293-302.

Jirakulaporn T, and Muslin AJ (2004). Cation diffusion facilitator proteins modulate Raf-1 activity. *J Biol Chem* 279: 27807-27815.

Johnson MP, LM Haupt, and LR Griffiths 2004 Locked nucleic acids (LNA) single nucleotide polymorphism (SNP) genotype analysis and validation using real-time PCR. NAR 32: e55.

Krens SFG, He S, Lamers GEM, Meijer AH, Bakkers J Schmidt T Spaink HP, Snaar-Jagalska BE (2008). Distinct functions for ERK1 and ERK2 in cell migration processes during zebrafish gastrulation. *Developmental Biology* 319: 370-383.

Lichten, L.A., Cousins, R.J. (2009). Mammalian zinc transporters: Nutritional and physiologic regulation. *Annu. Rev. Nutr*, 29:153-176.

Nasevicius A and Ekker SC (2000). Effective targeted gene “knockdown” in zebrafish. *Nat Genet.* 26:216-220

Palmiter RD and Findley SD (1995). Cloning and functional characterization of a mammalian zinc transporter that confers resistance to zinc. *Embo J* 14(4), 639-649.

Pickart MA, Klee EW, Nielson AL, Sivasubbu S, Mendenhall EM, *et al.* (2006). Genome-wide reverse genetics framework to identify novel functions of the vertebrate secretome. *PLoS One* 1: e104. doi:10.1371/journal.pone.0000104.

Stemple DL (2004). TILLING- a high-thoroughput harvest for functional genomics. Nature reviews (*Genetics*), 4:1-5.

Thiese B and Thisse C (2004) Fast Release Clones: A High Throughput Expression Analysis. ZFIN Direct Data Submission.

Wang X, Wu Y, Zhou B (2009). Dietary zinc absorption is mediated by ZnT1 in *Drosophila melanogaster. FASEB Journal* 23: 2650-2661.

FIGURES

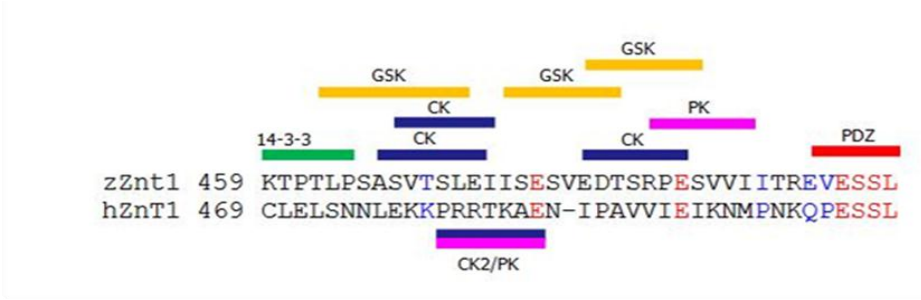


Fig 1: ClustalW multiple sequence alignment between zebrafish and human of the last 40 amino acids of the C-terminal of ZnT1 showing potential phosphorylation sites as predicted by ELM search

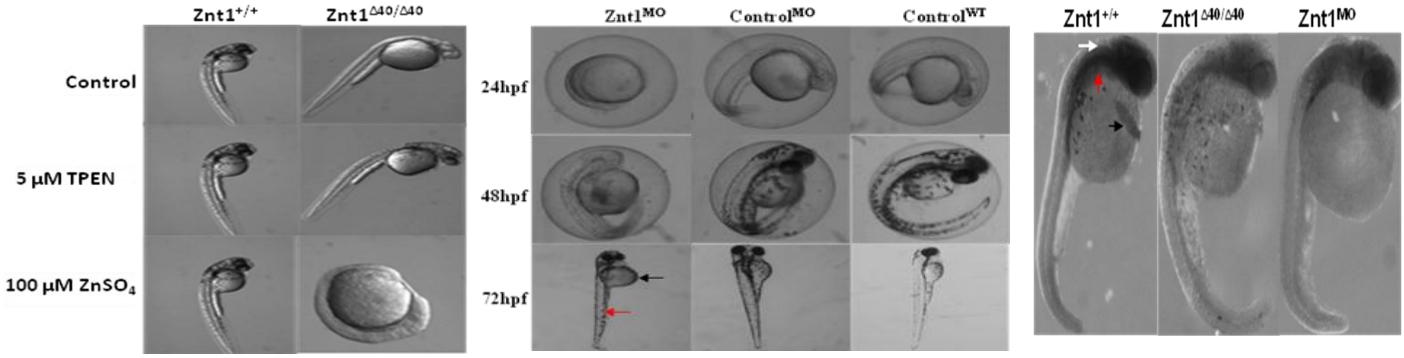


Fig 2: (A) Phenotype of Znt1 mutant embryo ($znt1^{\Delta 40/\Delta 40}$) and **(B)** Znt1 morphant embryo ($Znt1^{MO}$) showing showing morphogenetic difference in the stage of development (i.e. delayed development) as compared to wild-type ($znt1^{+/+}$). **(C)** Znt1 gene expression showing low expression in homozygote and morphant embryos compared to wild-type (arrows).

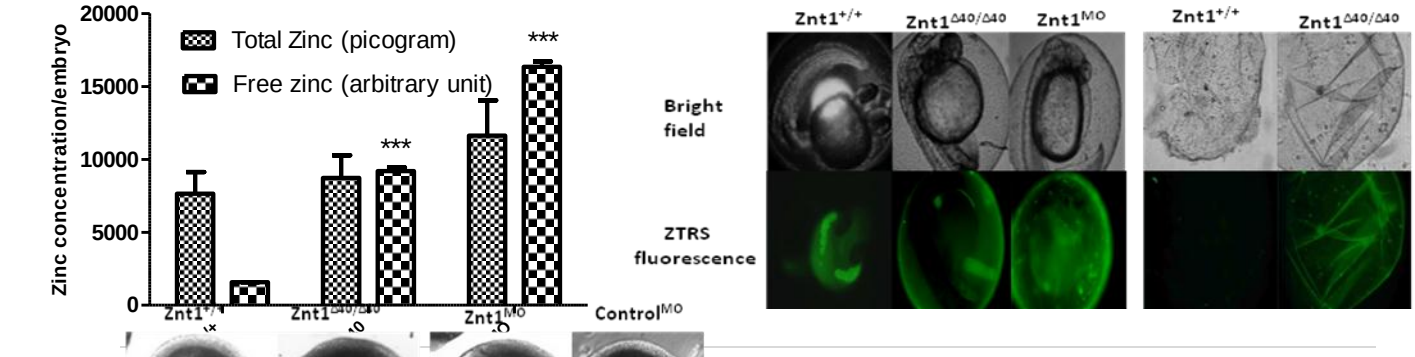
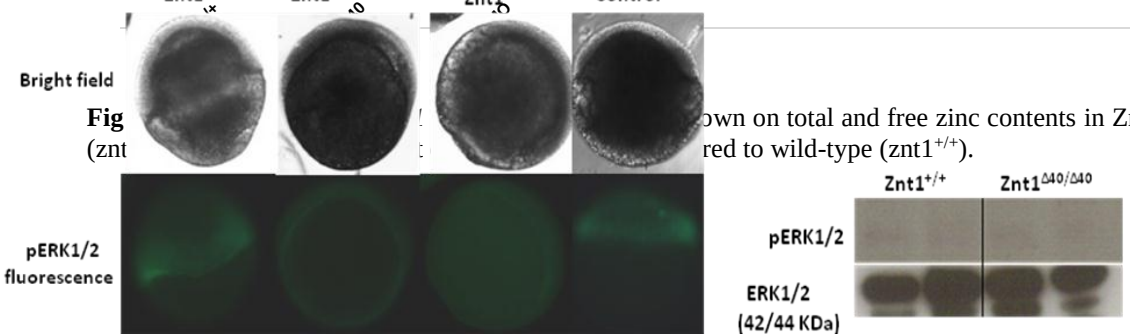


Fig own on total and free zinc contents in Znt1 homozygote mutant (znt red to wild-type ($znt1^{+/+}$).



MOLECULAR DETECTION OF BOVINE TRYPANOSOMIASIS IN SOME PARTS OF CENTRAL SENATORIAL ZONE OF PLATEAU STATE-NIGERIA

[1]Joana G. Peter,[2] E.I. Ikeh, [1]Dinchi J. Tyem,[1]Joannis T.M, and [1]Bitrus Y.

1. National Veterinary Research Institute, Vom
2. University of Jos, Department of Medical Laboratory Science

Correspondence: jaypee1410@gmail.com +2340833892259

INTRODUCTION

Trypanosomes are flagellates protozoa that belong to the genus Trypanosoma. They cause diseases that are generally referred to as trypanosomiasis. These protozoa can infect all classes of vertebrates (Connor and Van den Bossche, 2004). African animal trypanosomiasis (AAT) is a tsetse transmitted disease affecting livestock in Sub-Saharan Africa, (Cheneret *et al.,* 2006). In Nigeria, trypanosomiasis is probably one of the most important diseases of cattle, and one of the predominant obstacles to profitable animal production. The control of African animal trypanosomiasis is based primarily on chemoprophylaxis and chemotherapy. Therefore, in order to design national control strategies, knowledge on the distribution of tsetse flies and detection of trypanosome species/subspecies across the country is important. The latter requires methods for sensitive and specific detection of the trypanosomes. Jos Plateau offers a very suitable climate for livestock production especially with the presence of Integrated Diary Farm (IDF) and the National Veterinary Research Institute which adopted Friesian breeds to improve local breeds of cattle. Gathering of accurate data regarding the diagnosis of trypanosomiasis is crucial for the development of good treatment and control strategies. The diagnosis of trypanosomiasis including carrier status has been improved by the development and application of DNA based techniques such as PCR, which is very sensitive and effective method for the detection of chronic stage or pre-patent period of disease (Davila, 2005). Killed tyrpanosomal DNA does not remain in the blood for more than 24-48 hours, thus PCR based assay helps in the detection of only active infections after the drug therapy (OIE, 2003).

Seminar presented 25th June, 2015 at NVRI Auditorium

The aim of the study is to determine the prevalence of the disease in the study areas using the polymerase chain Reaction, and to characterize the *Trypanosoma* species.

MATERIALS AND METHODS

Study Area

The study was conducted in Bokkos Local Government Area of Plateau State. It is made up of 8 districts; namely, Bokkos, Daffo, Sha, Mushere, Manguna, Richa, Toff and Kamwai. It is located between 9^0 18'00"N and 9^0 00'00"E. It has a total area of 1682km^2 and a population of 178,454 as at the 2006 census.

Ethical Approval Statement

The study was carried out with the full approval of cattle keepers, the Plateau state ministry of Agriculture, the National Veterinary Research Institute, (NVRI) and the Nigerian Institute for Trypanosomiasis Research (NITR) Vom.

Sample collection

Blood samples were collected from cattle in the rural areas of the local government area which have high cattle population. Five (5) militres of blood were collected from the jugular vein using 10mls syringe and was transferred into bijou bottles containing EDTA salts and packaged in flasks containing icepack and were transported to Parasitology laboratory of National Veterinary Research Institute Vom.

Molecular Screening for Bovine Trypanosome

DNA Extraction

Total DNA was extracted from the buffy coat using the QIAmp®DNA mini kit according to manufacturer's specification. DNA was extracted from Buffy-coat layer of all the samples.

PCR Amplification and DNA probe analysis.

The PCR amplification of the samples was performed in a 50µl reaction in micro centrifuge tubes.

The primer sequences used were as follows:

Kin1 (reverse) 5'- GCG TTC AAA GAT TGG GCA AT-3'
Kin2 (forward) 5'-CGC CCG AAA GTT CAC C-3'

Gel Electrophoresis

Eight micro litres (8µl) of PCR amplicons were electrophorosed in a 1.5% agarose gel stained with ethidium bromide in the presence of 2µl of gel loading buffer (Fermentas®). Positive and negative controls were included. The electrophoresis was carried out at 100volts for 60minutes.

The electrophosed protein bands were read with the aid of a transilluminator.

RESULT

A total of 110 samples were collected from Bokkos and Daffo districts of Bokkos local government area of Plateau state. Out of the total cattle examined 30(27.27%) were males with 1(3.33%) positive; and 80(72.72%) females with 4(36.36%) positive. The Overall prevalence rate obtained was 5(4.54%) out of which 3(2.72%) were *Trypanosoma congolense* and 2(1.81%) were *Trypanosoma brucei*. Figures 1-3 shows the gel with amplification of samples and controls.

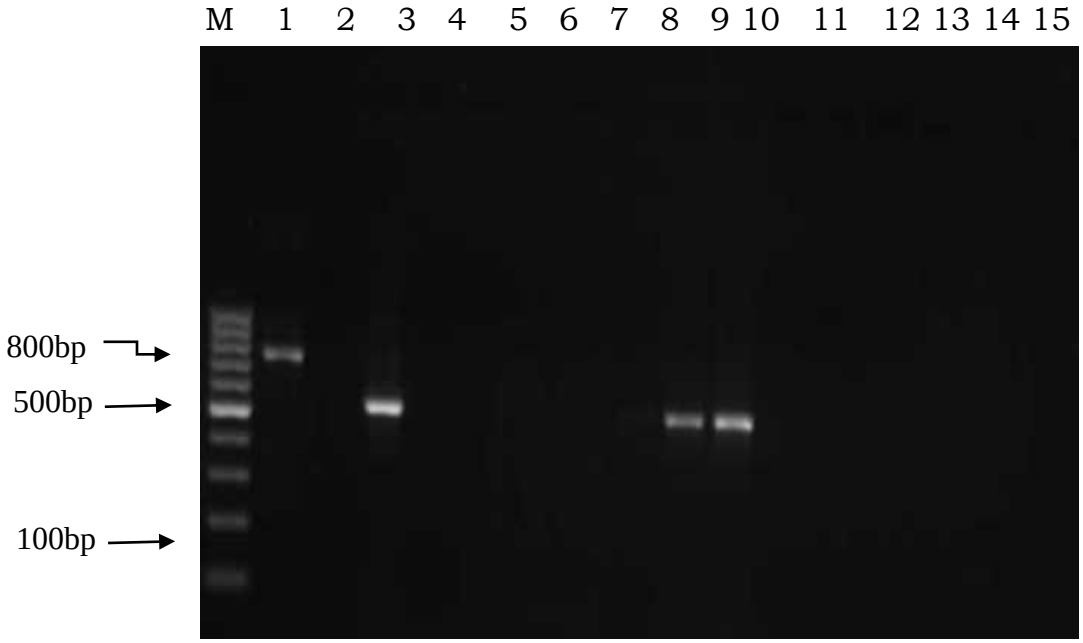


Figure 1: 1.5% Agarose gel electrophoresis of PCR products using kin 1 & kin 2 specific primers. Lane M: 100bp DNA molecular marker

(Fermentas®).Lane 1-*T.congolense forest (780bp)* Lanes 3&9 - *T. brucei* (540bp) Lanes - 10 & 15 are the positive and negative controls respectively.

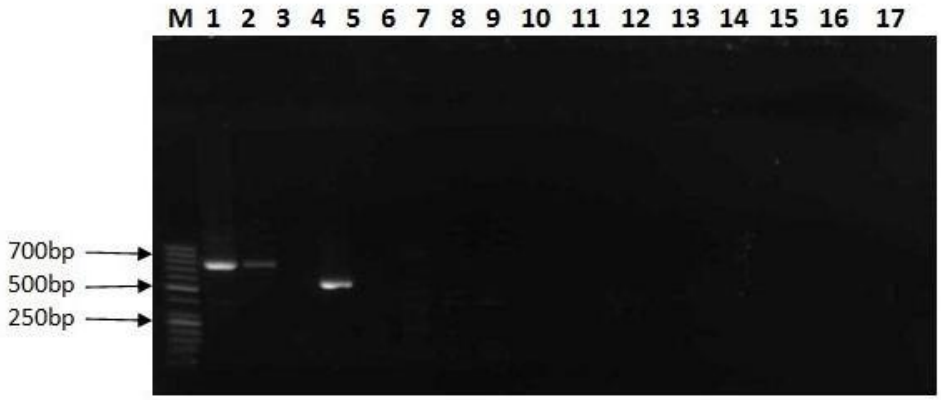


Figure 2: 1.5% Agarose gel electrophoresis of PCR products using kin 1 & kin 2 specific primers. Lane M: 50bp DNA molecular marker (Fermentas®). Lanes 1&2 – *T. congolense Kenya* (680bp). Lanes 4 & 17: Positive and Negative controls respectively.

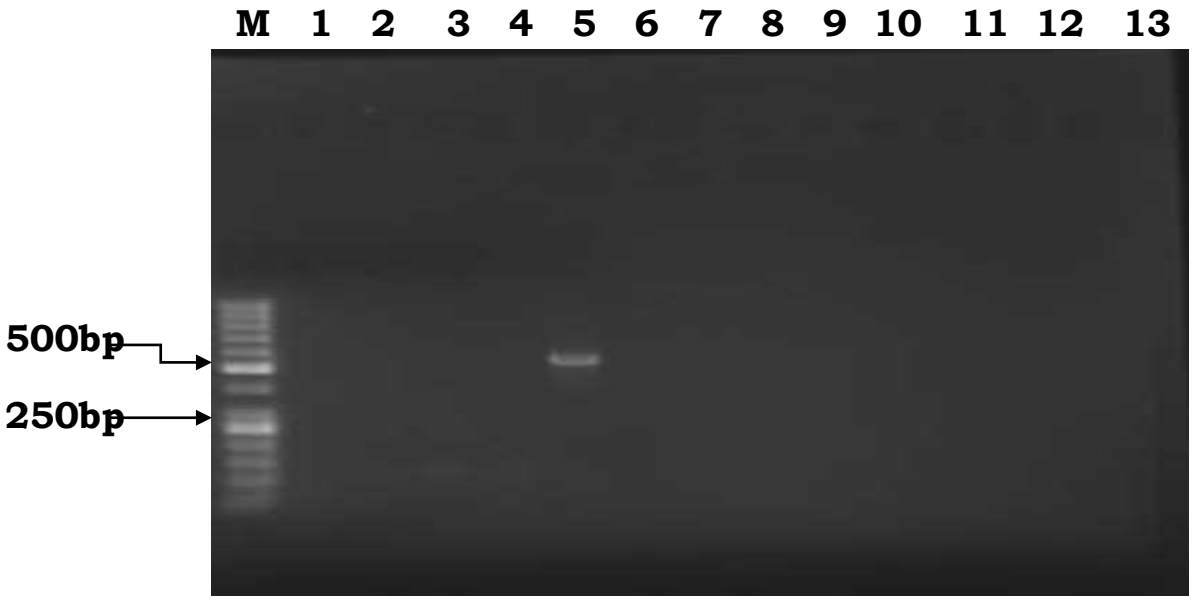


Figure 3: Absence of bands using specific primer for *Trypanosoma vivax.* Lane 5 is control.

DISCUSSION

Molecular methods for the species-specific identification of trypanosomes have been available for nearly 10 years (Masake *et al.*, 1994, Majiwa *et al.*, 1993, 1994). Despite this, their application in disease control and research programmes in the field has been limited to a handful of studies on cattle like that of Clausen *et al.* (1998). The PCR characterised *Trypanosoma brucei*, the savannah and the riverine forest of *Trypanosoma congolense* respectively. These strains are the types circulating in Daffo districts of Bokkos local government area. The application of DNA techniques provides much improved levels of sensitivity, such as the possibility of detection of individual organisms in samples of whole blood. The prevalence of *trypanosoma* species was generally higher in males 3(2.72%) than females 2(1.82%) possibly due to the fact that males are kept much longer for crossing and also the bulls are always in the fore front serving as a security to the flock as they go for pasture. This could possibly make the vectors come in contact with the bulls first. The absence of the parasites in young animals as compared to adults can be attributed to restrict grazing of young animals which tends to reduce their chances of contact with the vectors of the disease.

Trypanosoma congolense accounts for most of the infection, followed by *Trypanosoma brucei.* This is in contrast to the work done by Fajinmi, *et al.*, (2011). He observed that *Trypanosoma vivax* accounts for most of the infection in cattle at Sokoto Abattoir, Nigeria. The result obtained in this study is in agreement with the work done by Majekundun *et al.,* (2013) who detected a prevalence rate of 27.7% infection due to *T. congolense* in the Jos–Plateau. The specie most detected in this study *T. congolense* is in agreement with the result obtained by Michael *et al.*, (2013); He reported a prevalence rate of 48.7% due to *T. congolense* infection covering the northern and southern parts of the country. The present findings are also in consonance with the reports of Ogunsanmi *et al.* (2000) who reported a higher incidence of *T. congolense* in a survey carried out in South-western Nigeria.

CONCLUSION

This study has shown that PCR can be used effectively to study and validate carrier status of animal trypanosome infection. This study also suggests that *T. congolense* is the most prevalent species in the study area.

REFERENCES

Cherenet, T., Sani, R.A; Speybroeck, N., Pamandam, Nadzr, J.M., Vanden S., and Bossche, P.A (2006) Comparative longitudinal study of bovine Trypanosomiasis in tsetse-free and tsetse-infested zone of the Amhara Region, North-West Ethopia, *Veterinary Parasitology*, 140:251-258.

Clausen, P.H.,Wiemann A., Patzelt R., Kakaire D., Poetzsch C., Pergrine A., Mehlitz D.(1998). Use of a PCR assay for the specific and sensitive detection of Trypanosoma Spp. in naturally infected dairy cattle in peri-urban Kampala, Uganda. *Annals of NewYork Academy of science*, 849:21-31.

Connor, R.J., and Van den Bossche, P., (2004). African animal trypanosomiasis In: Coetzer, J.A.W., Justin, R.C. (Eds), Infectious diseases of livestock 2nd edition. Oxford University Press 1, 251-296.

Davila, A.M.R. (2005). The use of ITSI rDNA PCR in detecting pathogenic African trypanosomes, *Parasitology Research* 95:186-192.

Fajinmi, A.O., Faleke O.O., Magaji, A.A., Daneji A.I and Gweba (2011). Presence of Trypanosome species and Determination of Anaemia in Trade cattle at Sokoto Abbatoir, Nigeria. *Research journal parasitology,* 6: 31-42.

Majiwa, P.A.O., Maina, M., Waitumbi, J.N., Mihok, S., and weygarth, E. (1993). T*rypanosoma* (Nannomonas) *congolense:* Molecular characterisation of a new genotype from Tsavo, Kenya, *Parasitology* 106:151-162

Majiwa, P. A., Thatthi R., Moloo S.K., Nyeko J.H., Otieno L. H., Maloo S(1994). Detection of trypanosome infections in the saliva of tsetse flies and buffy-

coat samples from antigenaemic but aparasitaemic cattle, *Parasitology* 108: 313-322.

Masake,R.A., Nantulya V.M., Pelle R., Makau, J.M, Gathuo, H., and Ole-iYoi,O.K(1994). A species-specific antigen of *Trypanosoma* (Dutonella) *vivax* detectable in the course of infection is encoded by a differentially expressed tandemly reiterated gene. *Molecular Biochemistry* and *Parasitology* 64: 207-218.

Majekodunmi, A.O., Fajinmi A, Dongkum C, Picozzi K, MacLeod, E, Thrusfield, M.V, Alexandra, P.M, Welburn S.C. (2013) Social factors affecting seasonal variation in bovine trypanosomiasis on the Jos Plateau, Nigeria. *Parasites & Vectors* 6: 293

Michael, I. Takeet., Benjamin O. Fagbemi., Marcos De Donato., Abdulmojeed Yakubu., Hectorina E., Rodulfo.,Sunday O. Peters., Matthew Wheto., and Ikhide G. Imumorin. (2013) Molecular survey of pathogenic trypanosomes in naturally infected Nigerian cattle. *Research in Veterinary Science* 94: Pg 555-561

Ogunsanmi, A.O., Ikede, B.O., Akpavie, S.O., (2000). Effect of management, season, vegetation zones and breed on the prevalence of bovine trypanosomosis in Southwestern Nigeria. *Israel Journal of Veterinary Medicine* 55(2)

OIE: Terrestrial Animal Health code. Institute for International Cooperation in AnimalBiologics,(2003).Trypanosomiasis, www.who.int/tdr/publications/pdf/tsetsetraps.pdf

METHODS FOR THE IDENTIFICATION OF MYIATIC FLIES IN DOGS IN VOM, JOS-SOUTH LGA, PLATEAU STATE

Onovoh EO[1], Nwobu GO[2], Ogo IN[1], Peter JG[1], Onovoh EM[3], Bot CJ[4], Salihu AA[4]

1. Parasitology Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria.
2. College of Health Sciences, Igbinedion University Okada, Edo State, Nigeria
3. Federal College of Veterinary and Medical Laboratory Technology, Vom, Plateau State, Nigeria
4. Veterinary Clinic, Federal College of Animal Health and Production Technology, Vom, Plateau State, Nigeria.

Correspondences: emmaonovoh@gmail.com . +2348065541363

INTRODUCTION

Myiasis is a zoonotic disease caused by dipterous fly larvae feeding on the host's necrotic or living tissue. It is the infestation of the living human and vertebrate animals with dipterous larvae, which at least for a period feed on the host's dead or living tissue, liquid body substances, or ingested food (Zumpt, 1965).

Myiasis was first described by Hope in 1840 (Hope, 1840). It is a term applied to the infestation of live humans and other vertebrate animals with the larvae (maggots) of dipteral (two winged) flies. When open wounds are involved, the myiasis is known as traumatic and when boil-like, the lesion is termed furuncular (Veraldi, *et al.,* 1993).

Cutaneous myiasis is a temporary parasitic infestation of the skin of human and other vertebrates by larvae, the immature stage (maggots) of flies (Imam *et al.*, 2005). Myiasis can be accidental, as when fly larvae occasionally find their way into the human body, or facultative, when fly larvae enter living tissue opportunistically after feeding on decaying tissue in neglected, malodorous wounds. Myiasis can also be obligate, in which the fly larvae must spend part of their developmental stages in living tissue. Obligate myiasis is true parasitism and is the most serious form of the condition. Dogs and small rodents are a particularly important reservoir for the parasite (Ugwu and

Nwadiaro, 1999). The flies that cause furuncular myiasis include *Cordylobia anthropophaga* (tumbu fly, in sub-Saharan Africa) *Cordylobia rhodaini* (Lund fly, found in the rainforest areas of tropical Africa) and *Dermatobia hominis* (human botfly, which is endemic in Central and South America). As the modern, rapid international travel increases these myiatic infestations are now encountered outside these endemic regions (Imam *et al.*, 2005). Improper disposal of carcasses of both animals and man, surface effluents, faeces and other decomposing materials which man and domestic animals are exposed to have been identified as breeding sites for myiasis causing flies (Ogo *et al.*, 2005). Myiasis is a serious problem for the livestock industry, causing severe economic losses worldwide. Although infestation by fly larvae is much more prevalent in animals, it is a relatively frequent occurrence in humans in rural, tropical and subtropical regions (Adisa and Mbanaso, 2004). In humans, subcutaneous myiasis is caused by the larvae (maggots) of *Cordylobia anthropophaga* in sub-Saharan Africa, and *Dermatobia hominis* in Central and South America. Female *D. hominis* flies attach their eggs to mosquitoes or muscoid flies, which deposit them on warm-blooded hosts (e.g. cattle, humans); body warmth then triggers the larvae to 'hatch' and penetrate the host's skin. The larvae develop subcutaneously over 6-12 weeks through three stages, producing furunculoid lesions with central pores through which they breathe via spiracles and excrete serous/sero-purulent gut secretions. Left alone the third-stage larva will eventually leave the host and pupate in the soil to emerge as an adult fly in 1-3 months.

The eggs of Cordylobia species are however, deposited on the soil or wet and soiled clothes hung outside for drying. The hatched larvae invade unexposed skin of the body (buttocks, trunk, breasts, the limbs and penis) in contact with the wet clothes. Mature larvae then emerge from the host and pupate in the soil (Imam *et al.*, 2005). A red papule forms at the site of penetration and gradually enlarges; the lesions develop into a furuncle. The furuncle's aperture opens, permitting fluids containing blood and waste products of the maggot to drain (Geary *et al.*, 1999)

Cutaneous myiasis caused by the tumbu fly should be suspected when a patient who has just spent time in endemic area presents with ulcers or boil-like sores. A definitive diagnosis can only be made when the larvae are found.

They should be removed and allowed to develop into adult flies for identification purposes (John and Petri, 2006). This paper describes a cultural method in addition to standard method for the diagnosis and identification of myiasis causing flies.

MATERIALS AND METHODS

The study was conducted in Vom which is located within latitude 09^0 44' N, longitude 08^0 47' E and at an altitude of 1293.2 meters above sea level in the Jos South Local Government Area of Plateau State, in the Savannah region of Nigeria. Relative humidity range from 22% in January to 78% in July/August

Extraction of Larvae

Samples were collected from dogs attending the Veterinary Clinic, Federal College of Animal Health & Production Technology, Vom. Dogs showing symptoms of myiasis only were selected for this study. The larvae were harvested by the method described by Olumide, (1994). These were carefully expressed from the dogs by gentle pressure on the sides of the boil-like lesion and with the use of forceps; the larvae were pulled out and placed in Petri dishes. The expressed larvae were immediately taken to the Entomology laboratory, National Veterinary Research Institute Vom, where the third instars larvae were carefully identified and separated for in-vitro culture and posterior spiracular plating. Larvae for posterior spiracular plating were placed in universal bottles containing 10% formol saline for dissection.

Demonsration of Posterior Spiracular Plate

Posterior spiracular plating was carried out to identify the species of fly. Each of the larvae was heated in 10% potassium hydroxide solution for 3 minutes at 56^oC. The posterior part was cut with the aid of a sharp scalpel blade at about 1mm from the end. The tissues around the spiracular plate were teased out leaving the chitinous posterior spiracular plate on the slide. This was dehydrated with different changes of ascending grade (70%, 90%, and 100%) of ethanol. It was then cleared with xylene, mounted with DPX and examined under the microscope using 4x and 10x objectives (Service, 1996).

Culture of the Third Stage Larvae

The third instars (L3) larvae collected from dogs were placed on fine sterile sand collected in wide mouthed medium sized plastic containers. The larvae

were allowed to burrow into the sand. These containers were carefully and securely covered with net. The set up was then placed in the insect proof experimental room and allowed to pupate and for the adult fly to emerge. Daily temperature and humidity (ranging from 22% in January – 78% in August) were monitored.

RESULTS

A total of 325 larvae were extracted from 58 dogs, and were all indentified to be *Cordylobia anthropophaga* with the demonstration of the characteristic posterior spiracles. Out of this number, 138(42.5%) larvae were from 23(39.7%) male dogs, while 187(57.5%) were from 35(60.3%) female dogs (Table1). By age group, the infestation was more in puppies (0 – 6 months) having 164 (50 .5%) larvae extracted from 34(58.6 %) dogs. Those between 7 – 12 months had 60 (18.5%) larvae extracted from 15 (25. 9%) dogs and the last group >12 months had 101(31.0%) larvae extracted from 9(15.5%) dogs

The result, by the cultural method also showed the flies responsible for the dog cutaneous myiasis were of *Cordylobia anthropophaga* species. 13 out of the 15 carefully selected 3rd instar larvae which were subjected to in-vitro culture, hatched into the adult flies with the characteristic morphological diagnostic features as shown in the Appendix.

Results showing age and sex of dogs in relation to number of larvae extracted

		Age of dogs (Months)/Number of larvae extracted							
		0-6 months		**7-12months**		**>12months**		**Total**	**Total No of**
		No of dogs (%)	**Larvae Extracted (%)**	**No of dogs (%)**	**Larvae Extracted (%)**	**No of dogs (%)**	**Larvae Extracted (%)**	**No of dogs (%)**	**Larvae (%)**
Sex									
	Male	13(38.2%)	79(48.2%)	6(40.0%)	23(38.3%)	4(44.4%)	36(35.6%)	**23**(39.7%)	**138**(42.5%)`
	Female	21(61.8%)	85(51.8%)	9(60.0%)	37(61.7%)	5(55.6%)	65(64.4%)	**35**(60.3%)	**187**(57.5%)
		34(58.6%)	**164(50.5%)**	**15(25.9%)**	**60(18.5%)**	**9(15.5%)**	**101(31.0%)**	**58(100%)**	**325(100%)**

DISCUSSION

The PCR or molecular approach to the identification of myiatic flies remains the most sensitive method, but the cost and the fact that it is not done routinely makes the cultural method most acceptable in a lean economy.

This results show that all the larvae 325(100%) extracted from infested dogs sampled were identified by both posterior spiracular and cultural methods to be *Cordylobia anthropophaga.* This is in line with the findings of Ogo *et al.* (2009) and Ojemudia *et al.* (2010) but contrasts with that of Uva and Onyeka (1998) who separated *Oestrus ovis* and *Gastrophilus nasalis* in other domestic animals different from dogs within the same area. *Cordylobia anthropophaga has* also been incriminated in humans as is evident in the works of Ojemudia *et al* (2010) which showed that the infestation is found in humans as well.

Myiasis of domestic and wild animals have been considered issues of economic and public health, along side most arthropod/arthropod transmitted diseases, since ancient times, due to the significant damage they cause to the hides and skin of livestock, and also the fact that some of these larvae parasitize humans (Baily and Moody, 1985; Otranto and Stevens, 2002).

CONCLUSION

Dogs are close companions of humans and serve as reservoir host for the larvae of *C. anthropophaga.* It is therefore necessary to inform local residents and especially dog owners of the need to remove rubbish around homes regularly, dispose of faeces, carcases and decomposing materials all of which serve as breeding sites for this fly.

More attention should be paid to the presence of this fly now that it can be easily recognized from the result of the culture.

ACKNOWLEDGEMENTS

We are grateful to the EDVR and the management of NVRI Vom, for sponsoring this research work, as well as Mr. Pam Dambiong and other staff of Parasitology Division and the Vet clinic FCAH&PT Vom.

REFERENCES:

Adisa, Charles and Mbanaso, Augustus (2004). Furuncular myiasis of the breast caused by the larva of the tumbu fly (*Cordylobia anthropophaga*). *BMC Surgery* 4:5.

Baily, G.G., A.H. Moody (1985). Cutaneous myiasis caused by *C. anthropophaga* acquired in Europe. *Bri. Med. J.* 290, 1473-1474.

Geary, Merilyn J; Bernard J. Hudson; Richard C. Russell and Andrew Hardy (1999). "Exotic myiasis with Lund's fly (*Cordylobia rodhaini*)" *Medical Journal of Australia* 171 (11-12): 654–5.

Hope F.W.; (1840). On insects and their larvae occasionally found in human body. *Trans R Soc Entomol*, 2:256-271.

Imam, A.M., Musa, H.A. and Nugud, O.O. (2005).Cutaneous Myiasis: Report of two cases. *Sudanese Journal of Dermatology*; 3(1): 48-50.

John and Petri (2006). Medical parasitology*: 9th Edition. Missouri: Saunders Elsevier*, p. 328-334.

Ogo I.N., Mwansat G.S. Jambalang A. Ogo, M.F., Onovoh E., Ogunsan, E.A., Dogo, G.I., Bayingyi, S., Odoya, E.M., Chukwu, O.O.C., Yako, A.B., and Iyama, P.U. (2005). Retrospective study on the prevalence of canine myiasis in Jos-South Local Government Area of Plateau State, Nigeria*, J. est. Dis. Vect. Mgt.* 6, 385-390.

Ogo, N.I., E. Onovoh, D.R. Ayodele, O.O. Ajayi, C.O. Chukwu, M. Sugun, I.O. Okeke (2009): Cutaneous canine myiasis in the Jos metropolis of Plateau State, Nigeria, associated with *Cordylobia anthropophaga*. *Vet. Arhiv* 79, 293-299.

Ojemudia (2010): Emergence of Zoonotic myiasis in Vom and Bukuru metropolis, Jos South LGA, Plateau State, Nigeria. *Global Health Science Journal, Vol. 3 No.1.*

Otranto, D., J.R. Stevens (2002): Molecular approaches to the study of myiasis causing larvae. *Int. J. Parasitol.* 32 (11), 1345-1360.

Olumide Y.(1994) Cutaneous miasis: a simple and effective technique for extraction of *Dermatobia hominis* larvae. *Int J Dermatol.*; 33:148-149.

Service MW (1996) Medical Entomology for students. London and New York: Chapman and Hall.

Uva, C.U.T., Onyeka J.O.A. (1998): Preliminary studies on myiasis in domestic animals slaughtered in Jos abattoir, Plateau State, Nigeria. *Afri. J. Nat. Sc.* 2, 111-112.

Ugwu, B.T and Nwadiaro, P.O. (1999) *Cordylobia anthropophaga* Mastitis mimicking Breast Cancer: Case Report. *East African Medical Journal* 76(2): 115–116.

Veraldi S.; Brusasco A; Suss; (1993) Cutaneous myiasis caused by *Cordylobia anthropophaga* (Blanchard). *Int J Dermat*, 32:184-182.

Zumpt, F (1965). Myiasis in Man and Animals in the Old World, *Butterworths, London, UK,* 267 pp.

Appendix

Collected larvae

Preserved Larvae

Larvae incubating in sand

Hatched *Cordylobia anthropophaga fly*

Characteristic Wing Vein

ENDOGENOUS RETROVIRUS CONTAMINANTS OF FELINE CELL LINES: IMPLICATIONS FOR VETERINARY VACCINE MANUFACTURE

Arowolo Oluwadamilola A

Viral Vaccines Production Division, National Veterinary Research Institute, Vom

Email: drdammy@gmail.com

INTRODUCTION

During evolution, the genomes of all animal species have been colonized by endogenous retroviruses (ERVs). These ERVs have integrated into the host cell germ line as a "provirus" that can be vertically transmitted between generations like any other Mendelian gene (Boeke and Stoye, 1997). All domestic cats have a replication-competent ERV, the RD-114 virus, incorporated into their genome (Baumann *et al.*, 1998; Okada *et al.*, 2011; van der Kuyl *et al.*, 1999).

RD-114 is a member of the Gammaretrovirus genus, whose members include feline and murine leukemia virus (Miyazawa *et al.*, 2010). The virus was first isolated in 1971 from human rhabdomyosarcoma cells that were transplanted into foetal cats (McAllister *et al.* 1972). However, RD-114 is considered to have originated in baboons due to its genetic similarity to baboon endogenous retrovirus. The *env* gene of RD-114 is almost identical to that of baboon endogenous retrovirus, and similar to those of betaretroviruses. The *gag-pol* genes however, are similar to gammaretroviruses (van der Kuyl *et al.*, 1999). The baboon endogenous retrovirus is thought to have infected an ancestral *Felis* species, with a de novo recombinant colonising the cat germ line before the Felis genus branched into separate species (van der Kuyl *et al.*, 1999).

Some feline cell lines such as Crandell-Rees feline kidney (CrFK) cells commonly used to grow feline and canine viruses express variable amounts of RD-114 (Baumann *et al.*, 1998), and a number of live-attenuated feline and canine vaccines produced using feline cell lines (Miyazawa, 2010; Yoshikawa *et al.*, 2011) and even non-feline cell lines (Yoshikawa *et al.*, 2012) have been found to be contaminated with the RD-114 virus. These vaccines are commonly used on millions of animals worldwide.

Seminar presented 9th July, 2015 at NVRI Auditorium

Completely knocking out the RD-114 provirus from feline cells is difficult since ERVs are usually integrated (in multiples) into the host cell genome. However, it may be possible to reduce the contamination of RD-114 virus by regulating the release of the virus from cells (Fukuma *et al.*, 2011).

Since the stability of the RD-114 virus in the presence of other viruses is uncertain, a major part of this study was to investigate if other feline virus infections interfere with RD-114 production and release from cells. The study aimed to detect the production and presence of RD-114 virus in culture fluids of three established feline cell lines using an optimized Product Enhanced Reverse Transcriptase (PERT) assay.

Examine the effect of virus infection of feline cell lines on the production and release of RD-114 virus, by infecting RD-114-producing and non-producing feline cell lines with three different feline viruses

METHODS

Cell culture

Three different feline cell lines were used in this study: the ID10 cell line, a clone of Crandell Rees Feline Kidney (CrFK) cells (Willett *et al.*, 1997), the FEA cell line, and the AH927 cell line. ID10 cells naturally release RD-114 virus into culture fluids (Baumann *et al.*, 1998) while the FEA and AH927 cell lines do not, even though they both have the RD-114 provirus incorporated into their cellular genomes (Okada *et al.*, 2011).

All cell lines were maintained in Dulbecco's Modified Eagle's medium (Life Technologies Ltd.,UK) supplemented with 10 % (v/v) heat-inactivated Foetal Bovine Serum (Hyclone, UK), 100 IU/ml Penicillin and 100 µg/ml Streptomycin (Life Technologies, Ltd., UK), subcultured into 25cm^3 culture flasks (Corning Inc.,USA) at a concentration of 10^5 cells/ml (or 1.4 x 10^6 cells/ml for ID10 cells infected with feline calicivirus (FCV) and incubated at 37 ˚C under 5 % CO_2 in a humidified chamber.

Virus Infection

Three feline viruses were used in this study: feline calicivirus (FCV), 4.1 x 10^6 $CCID_{50}$/ml; feline herpes virus (FHV), $10^{7.5}$ $CCID_{50}$/ml; and feline pox virus (FPV), $10^{4.5}$ $CCID_{50}$/ml. Seven 5-fold serial dilutions of the stock viruses were made, starting at 125 $CCID_{50}$ for FCV, 10^3 $CCID_{50}$ for FHV, and the original stock of FPV. 50 µl from each 5-fold virus dilution was infected into each cell

line. 100 µl aliquots of culture fluid were taken from each cell line before virus infection (day 0) and every 24 hours for 7 days after virus infection. Aliquots were stored at -20 ˚C until used for PERT assay. Culture fluid from uninfected cells were used as test control (NI-C), culture fluid from established ID10 cells used as positive control (PC) and distilled water used as negative control (NC).

Molecular Biology

PERT assay

To detect RD-114 virus, PERT assay (adapted from Pizzato *et al.*, 2009) was performed on culture fluid samples and controls in wells of a MicroAmp® fast optical 96-well reaction plate (Life Technologies Ltd., UK), allowed to run in a 7500 fast real-time PCR machine and analysed using the ABI 7500 software (Applied Biosystems).

DNA, RNA extraction and sequencing

Genomic DNA (gDNA) was extracted from ID10, FEA and AH927 cell pellets using the QIAamp DNA Blood Mini kit (Qiagen, UK). RD-114 virus was extracted and purified from culture supernatants of ID10 and AH927 cells using the QIAamp UltraSens Virus Kit (Qiagen, UK), and then reverse transcribed to complementary DNA (cDNA) using a Transcriptor High Fidelity cDNA Synthesis Kit (Roche). A partial RD-114 DNA segment was amplified from gDNA and cDNA using primers corresponding to a 500 bp portion of the *pol* gene, forward primer (5’ TCA TAA TTG ACC TAA AGC CCAC 3’) and reverse primer (5’ CTG AGT TGT GGG TCT TCA TTG 3’) and the KOD Hot Start DNA Polymerase Kit (Merck Chemicals Ltd., UK) in a GeneAMP PCR system 9700 Thermocycler (Applied Biosystems). The gDNA and cDNA *pol* PCR products were sequenced by GATC Biotech Ltd., London, and using the ABI 3700 automated capillary array sequencer respectively. Raw chromatograph data were analysed using Bioedit software.

RESULTS AND DISCUSSION

Cell culture

Virus-infected cells in culture showed cytopathic effects (CPE), usually from the 2nd day post-infection. The onset and degree of CPE observed depended on the type and concentration of virus infected. CPE ranged from cell rounding to empty spaces in the monolayer and eventually to floating dead cells. Uninfected cells did not show CPE and remained healthy until about the 6th day of culture when they started to age normally (figure 1).

PERT assay

PERT assay results were interpreted using the threshold cycle (Ct) value which is inversely proportional to reverse transcriptase (RT) enzyme activity (which in this case represents the quantity of RD-114 virus particles in the culture fluid samples). Results from samples obtained from ID10 cells and AH927 cells were similar. RD-114 RT activity was detected, which gradually increased to a peak level and then later decreased (figure 2). An increase in RT activity was noticed as cell population increased and a decrease in RT activity coincided with onset of CPE (in infected cells) or cell aging (in uninfected cells). There was no RD-114 RT activity detected in samples from FEA cells.

The feline viruses (FCV/FHV/FPV) introduced appeared to have no effect on RD-114 production/ replication from the feline cell lines under study. Instead, RD-114 production corresponded to the population of viable cells in culture (similar trend of Ct values in infected and uninfected cells). FEA cells, as expected, did not produce the RD-114 virus. However, RD-114 virus was detected in supernatants from AH927 cells which were previously assumed to be non-producers of the RD-114 virus.

Sequence analysis

RD-114 *pol* sequences from AH927 and FEA genomes showed only one nucleotide difference when compared with each other, but had 13 and 14 nucleotide differences respectively when compared with the published RD-114 molecular clone. The *pol* sequence from ID10 cells, however, was more similar to the RD-114 molecular clone having only 8 nucleotide differences (figure 3). This result also confirms the presence of the RD-114 provirus in the genome of the three cell lines, with the release of RD-114 virus particles into the culture fluid from ID10 and AH927 cells.

CONCLUSION

The production and release of RD-114 virus was found to increase with increase in cell number and to decrease with cell death resulting either from the normal aging process or viral cytopathic activity. This finding is expected; as RD-114 is an endogenous retrovirus with the feline cell genome responsible for its viral propagation. More copies of the virus will be produced when there is a higher volume of cellular genomic material, which in turn has increased as a result of increased cellular proliferation.

The results also imply that exogenous virus infections of feline cell lines have no apparent direct effect on the production and release of RD-114 into culture fluid. These viruses themselves utilize cellular materials for their own replication and do not seem to interfere with the propagation of RD-114.

Although we observe no apparent interaction between exogenous virus infection and endogenous RD-114 virus production and release from feline cell lines, the risk of using these cell lines in vaccine production still exists. RD-114 virus may not be pathogenic to feline hosts but cross-species transmission is unpredictable and chronic effects may occur in future. Further studies should be conducted to establish if RD-114 causes a chronic negative impact on cats and dogs. This is because RD-114 is a xenotrophic virus capable of productively infecting cell lines from other species (such as dogs and humans). A sodium-dependent neutral amino acid transporter, termed ASCT2, has been identified as the receptor for RD-114 virus on such cell lines (Okabe *et al.* 1973; Baumann *et al.* 1998; Miyazawa *et al.* 2010; Yoshikawa *et al.* 2012).

No problems have yet been reported following current vaccination procedures, as it is likely that low-level exposure to RD-114 does not induce a significant risk. Despite a low possibility of disease, it would be better to remove RD-114 from future vaccinations, and the most feasible method for now is to avoid using feline cell lines like CrFK cells that produce RD-114 virus in the manufacture of vaccines.

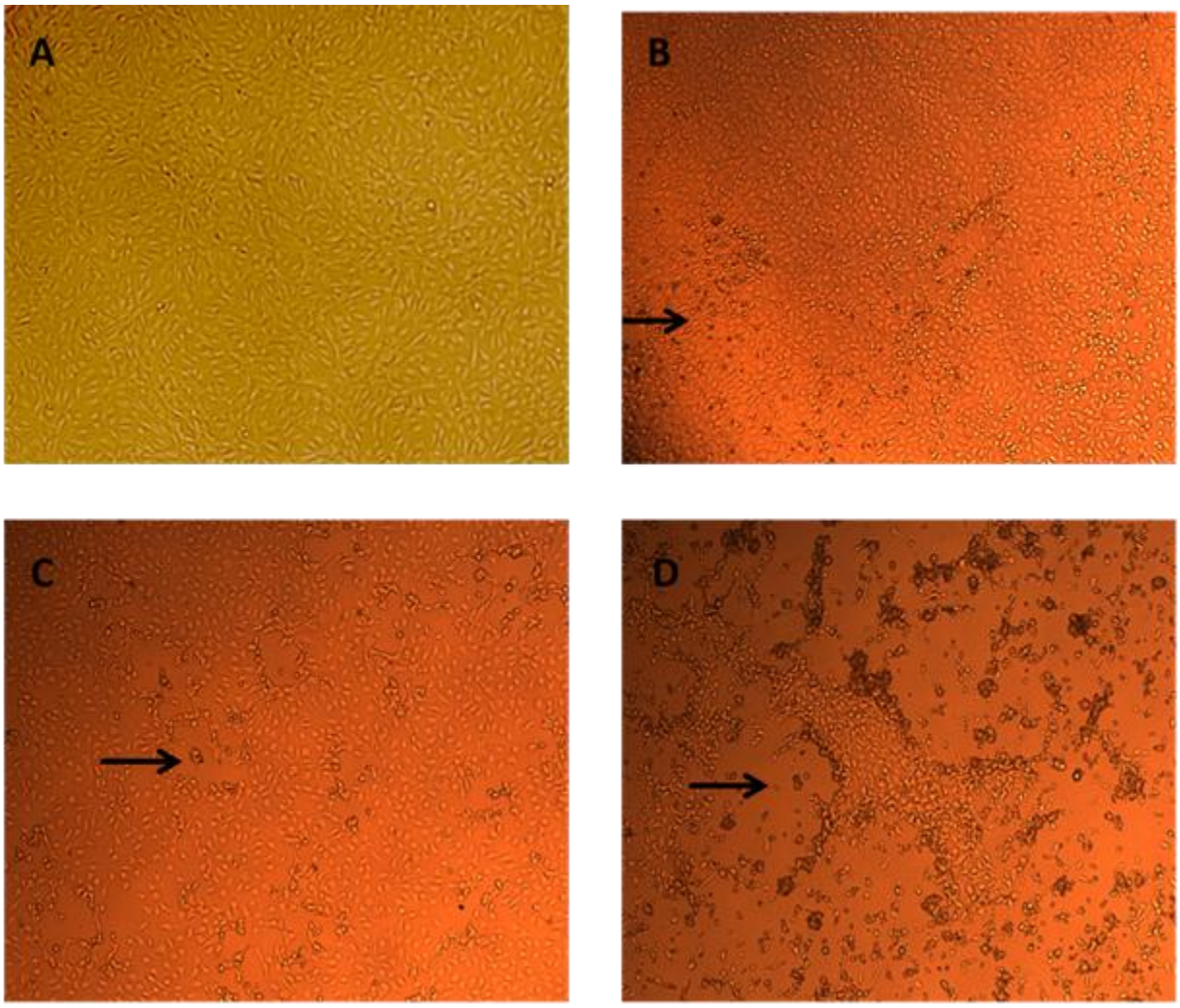


Figure 1: FEA cells (x 10) showing typical CPE from FCV infection at day 2 (B), FHV infection at day 4 (C) and FPV infection at day 6 (D) and confluent monolayer of uninfected cells (A). CPE was seen as cell rounding, vacant spaces in the cell monolayer, and floating cells (see arrows).

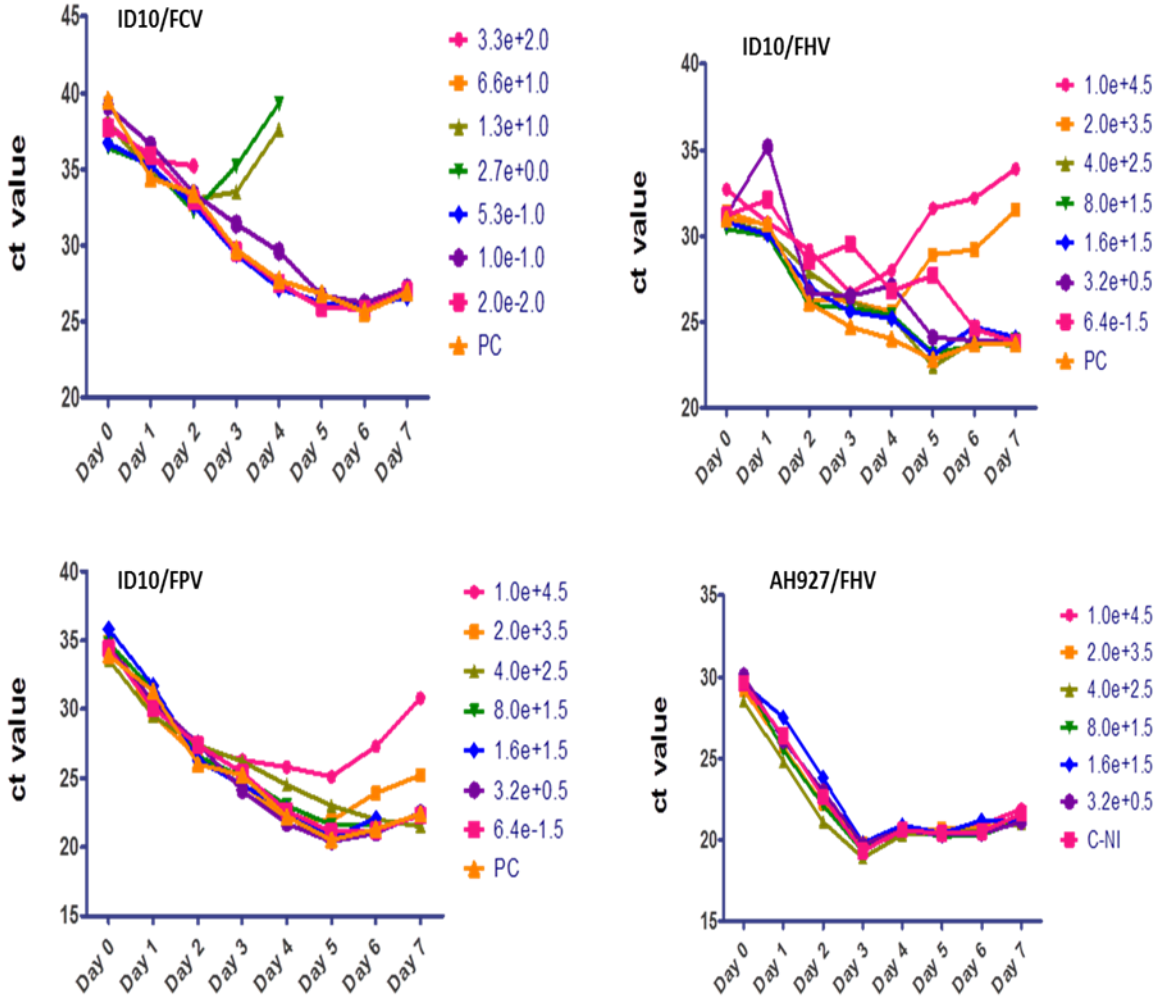


Figure 2: PERT assay of culture supernatants showing a similar trend in Ct values for ID10 and AH927 feline cell lines (infected and uninfected). Decreasing Ct values correspond to increasing RD-114 RT activity. Onset of CPE was marked by a sharp increase in Ct values, which implies lowered production of RD-114 virus/RT activity.

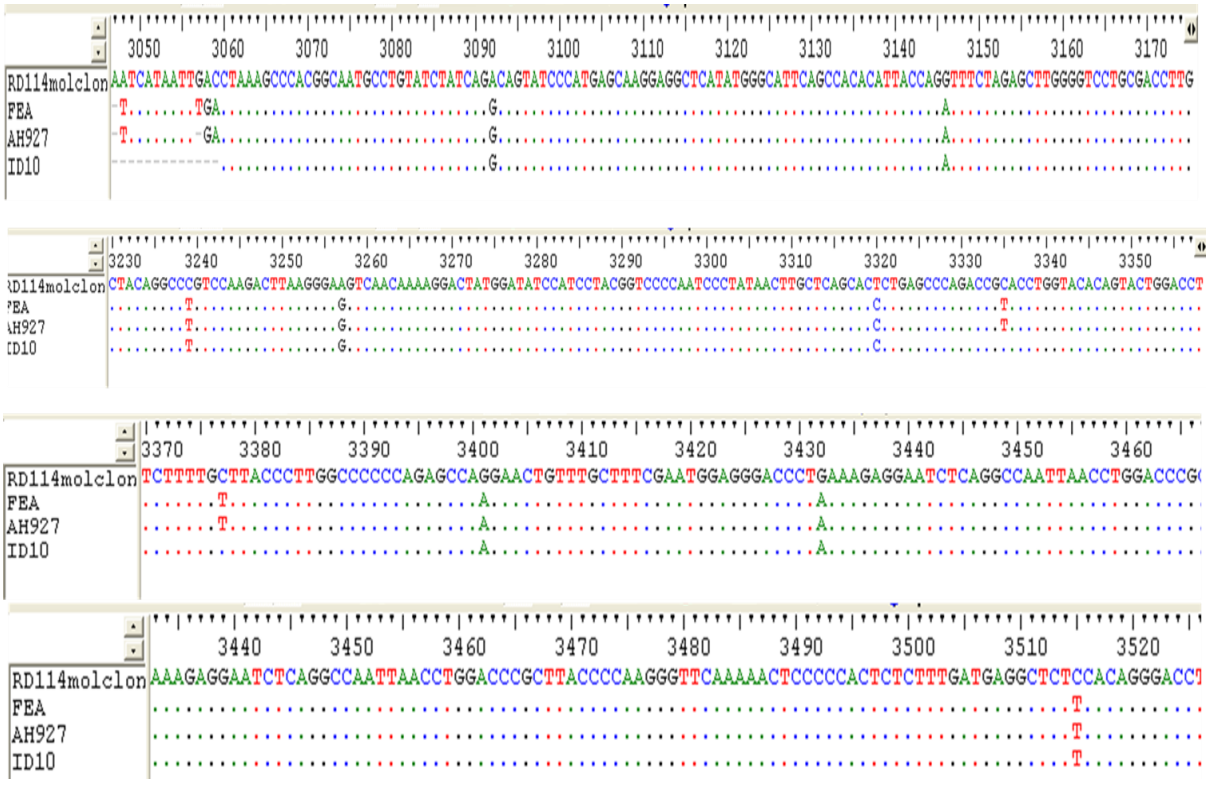


Figure 3: ID10, FEA and AH927 partial RD-114 pol sequence alignment with a known RD-114 virus molecular clone. The similarity confirms the presence of the RD-114 provirus in the genomes of these feline cell lines.

ACKNOWLEDGEMENTS

Drs Margaret J. Hosie, Ayman A. Samman, and Brian J. Willett of the College of Medical, Veterinary and Life Sciences, University of Glasgow who all supervised this work.

REFERENCES

Baumann, J.G., Gonzburg, W.H. and Salmon, B. (1998). CrFK Feline Kidney Cells Produce an RD114-Like Endogenous Virus That Can Package Murine Leukemia Virus-Based Vectors, *Journal of Virology*, 72(9), pp.7685-87.

Boeke, J. D; Stoye, J.P (1997). Retrotransposons, endogenous retroviruses and the evolution of retroelements, p. 343–436: In J. M. Coffin, S. H. Hughes,

and H. E. Varmus (ed.), Retroviruses. Cold Spring Harbor Laboratory Press, Plainview, NY

Fukuma, A., Abe, M., Urata, S., Yoshikawa, R., Morikowa, Y., Miyazawa, T. and Yasuda, J. (2011). Viral and cellular requirements for the budding of feline endogenous retrovirus RD-114, *Virology Journal*, 8(1), p.540

McAllister, R.M. and Nicolson, M. (1972). C-type virus released from cultured human rhabdomyosarcoma cells, *New Nature Biology*, 235(53), pp.3-6

Miyazawa, T., Yoshikawa, R., Golder, M., Okada, M., Stewart, H. and Palmarini, M (2010). Isolation of an infectious endogenous retrovirus in a proportion of live attenuated vaccines for pets, *Journal of Virology*, 84(7), pp.3690–4.

Miyazawa, T., (2010). Endogenous retroviruses as potential hazards for vaccines. *Biologicals : Journal of the International Association of Biological Standardization*, 38(3), pp.371–6.

Okabe, H., Gilden, R. V and Hatanaka, M., (1973). RD 114 virus-specific sequences in feline cellular RNA: detection and characterization. *Journal of Virology*, 12(5), pp.984–94

Okada, M., Yoshikawa, R., Shojima, T., Baba, K. and Miyazawa, T (2011). Susceptibility and production of a feline endogenous retrovirus (RD-114 virus) in various feline cell lines, *Virus Research*, 155(1), pp.268–73

Pizzato, M., Erlwein, O., Bonsall, D., Kaye, S., Muir, D. and McClure, M.O (2009). A one-step SYBR Green I-based product-enhanced reverse transcriptase assay for the quantitation of retroviruses in cell culture supernatants, *Journal of Virological Methods*, 156 (1-2), pp.1–7.

van der Kuyl, A.C., Dekker, J.T. and Goudsmit, J. (1999). Discovery of a new endogenous type C retrovirus (FcEV) in cats: evidence for RD114 being an FcEVGagePol/baboon endogenous virus BaEVEnv recombinant. *Journal of Virology*, 73(10), pp. 7994-8002.

Willett, B., Hosie, M., Shaw, A and Neil, J., (1997). Inhibition of feline immunodeficiency virus infection by CD9 antibody operates after virus entry and is independent of virus tropism. *The Journal of General Virology*, 78 (Pt 3), pp.611–8.

Yoshikawa, R., Sato, E and Miyazawa, T (2011). Contamination of infectious RD-114 virus in vaccines produced using non-feline cell lines. *Biologicals : Journal of the International Association of Biological Standardization*, 39(1), pp.33–7.

Yoshikawa, R., Sato, E. and Miyazawa, T., (2012). Presence of Infectious RD-114 Virus in a Proportion of Canine Parvovirus Isolates. *Journal of Veterinary Medical Science*, 74(3), pp.347–350.

SEROPREVALENCE OF INFLUENZA A ANTIBODIES IN PIGS IN JOS-SOUTH LOCAL GOVERNMENT AREA OF PLATEAU STATE, NIGERIA.

Ijomanta, O.J*[1], Okolocha, E.C[3], Bello, M[3], Kudi, C.A[4], Meseko, C[2], Nwosuh, C.I[2], Micheal, C.A[5]

[1]Viral Vaccines Production Division, NVRI, Vom
[2]Viral Research Department, NVRI, Vom
[3]Department of Veterinary Public Health and Preventive Medicine, ABU, Zaria
[4]Department of Veterinary Medicine, ABU, Zaria
[5]Federal Department of Veterinary and Pest control Services, Abuja.

Correspondence: jerrymanta@yahoo.com

INTRODUCTION

Swine influenza virus (SIV), a type A influenza, causes highly contagious viral respiratory disease that can have significant economic impact on affected pig herd (Oslen *et al.*,2008).

Swine influenza is a major concern to pig farmers as it can cause up to 100% morbidity and abortion in an infected herd (OIE, 2010).

Pigs are mixing vessels for re-assortment of avian, swine and human influenza viruses (Eileen and Bruce, 2008).

Occupational exposures among farmers and abattoir workers are contemporary health risks that can no longer be ignored (Meseko et al.,2010).

The chances of interspecies transmission can be enhanced by mixing of different species of animals as commonly practiced in semi intensive and rural farming in Nigeria where pigs, birds and other species inter-mingle (Awosanya *et al.*, 2014).

The aim of this study was to determine the sero-prevalence of influenza A antibodies and the subtypes in pigs in Jos South, Plateau State, Nigeria.

MATERIALS AND METHODS

The sample size used in this study was determined as described by Mugo (2008) using the formula below and a prevalence of 67% by Awosanya et al. (2014)

$N = Z^2pq/d2$

A sample size of 340 was arrived at but it was increased to 500 to increase precision. 350 pigs were sampled from slaughter slabs while 150 from piggeries in the study area using a systematic and purposive sampling methods.

Blood was collected from each pig using a 10 ml syringe with a 19 G needle from the anterior vena cava, into a sterile sample bottle. The blood was allowed to clot and the sera obtained from the blood stored at 4^oC until use.

The sera was used to determine the seroprevalence of swine influenza A antibodies using competitive ELISA influenza A kit (IDEXX, France).

While the subtypes were determined from the seropositive sera following ELISA, using the Haemagglutination inhibition test.

Statistical Analysis

Data obtained were summarized into tables using descriptive statistics. Chi-square and odds ratio were used to test for association between the variables. Values of $p < 0.05$ were considered significant.

RESULTS

Table 1: distribution of swine influenza antibodies among pigs in jos south LGA plateau state.

Variables	Number tested	Number (%) positive	X^2	P.	OR	CI
Sampling site			0.04	0.85	1.1	0.56-2.0
Slaughter slab	350	37(10.6)				
Pig farms	150	15 (10)				
Sex			0.1	0.73	0.9	0.5-1.61
Male	242	24(9.9)				
Female	158	28(10.9)				
Age			1.1	0.28	0.68	0.33-1.60
>1 year	420	41(9.8)				
<1 year	80	11(13.8)				
Location of farm			1.4	0.5		
Kuru	30	4(13.3)				
Vwang	50	3(6.0)				
Gyel	70	8(11.4)				

Table 1 shows the distribution of swine influenza antibodies among pig population in Jos South LGA, Plateau State. A total of 52 out of 500 (10.4%) samples were positive to swine influenza antibodies by the ELISA Test. More female than male pigs (10.9% vs 9.9%), and pigs less than 1 year than those above 1 year (13.8% vs 9.8%) were sero positive although the differences were

not statistically significant (p>0.05). similarly, the differences in sero prevalence was not significant (p>0.05) between the various sampling sites and farm locations (table1)

Table 2: Distribution of swine influenza subtypes among pigs in Jos South LGA

Variables	Number of ELISA positive	H1	H3
Sex:			
Male	24	9	1
Female	28	13	2
Source of sample:			
Slaughter slab	37	18	2
Pig farms	15	4	0

Two swine influenza subtypes; H1 and H3 were detected in the study (table 2). H1 subtype occurs more frequently than H3 subtype.

DISCUSSION

The results of the present study illustrate the existence of swine influenza A virus antibodies in piggeries and pigs slaughtered in slaughter slabs in Jos South Plateau State, Nigeria. The overall seroprevalence of 10.40% for swine influenza A in pigs in Jos South L.G.A recorded in this study is lower than the 29.4% and 67% recorded by Meseko (2013) and Awosanya *et al.* (2014), respectively, in the South Western part of Nigeria. The seroprevalence is however higher than the 4.6% recorded in Uganda by Kirunda *et al.* (2014), and also lower than the 39.1% recorded by Jeong *et al.* (2004) in South Korea. The study also showed that the seroprevalence of swine influenza A antibodies in pig slaughtered in slaughter slabs and piggeries in Jos South were similar. This could indicate exposure to influenza A strain viruses which could be from

pigs brought from other parts of the State or neighbouring States for slaughter in the slaughter slabs or from human or other animals especially avian species.

The non-significant association ($p > 0.05$) obtained from the study between swine influenza A antibody detection and the sex of pigs sampled indicates that sex does not influence swine influenza infection in pigs. This agrees with similar reports by Markowska-Daniel and Stankericius (2005) in Poland among sow and boars and Kirunda *et al*. (2014) in Uganda.

The non-significant association ($p > 0.05$) between detection of swine influenza A antibodies in pigs and the ages of the pig in Jos South L.G.A also indicates that age does not influence swine influenza infection. This contradicts the report of Jeong *et al*. (2004) who reported seroprevalence of antibodies to swine influenza according to ages as 47.2%, 19.6%, 36% and 66.7% for suckling, weaned, growing and finishing pigs and sows, respectively, as well as the findings of Takemae *et al*. (2011) and Kirunda. *et al*. (2014) who isolated influenza virus in young pigs and detected influenza antibodies in adult pigs only. This study showed that location does not significantly influence the detection of swine influenza A antibodies in piggeries sampled in Jos South L.G.A. The variation observed in their seroprevalences may be due to management system or herd size of the farms, as herd size have been reported by Kirunda *et al*. (2014) to influence swine influenza detection and in this study Kuru had the highest pig population followed by Gyel and Vwang, with similar patterns for their seroprevalence recorded there.

The study revealed that H1 and H3 subtypes of swine influenza A virus co-circulate in Jos South L.G.A. and this provides further evidence that pigs serve as intermediate host or mixing vessels, and emphasizes the importance of reinforcing swine influenza virus surveillance in every part of the country as recommended by Oladipo *et al*. (2013).

CONCLUSION

The study showed an overall seroprevalence of swine influenza antibodies in Jos south L.G.A of 10.40%.

There was no significant association between detection of swine influenza A antibodies and sex, age and location.

The study also showed that the subtypes of swine influenza found in the study area were H1 and H3.

RECOMMENDATIONS

Surveillance and monitoring of circulating swine influenza antibodies in other parts of the State and the country at large is important and should be carried out so as to establish the true prevalence of the disease in the country.

Public enlightenment and education should target populations that are not aware of the disease.

Broad and extensive studies of swine and other animals like domestic birds should be carried out in order to clearly understand the epidemiology of swine influenza viruses in Jos and Nigeria as a whole. This will help to establish the human-animal interface in the country.

REFERENCES

Awosanya, E.J.,Ogundipe, G., Babalobi, O., Omilabu, S. and Nguku, P. (2014). Prevalence and correlate of influenza 4 in piggery workers and pigs in two communites in Lagos, Nigerian. *Pan African Medical Journal*, Doi:10.11604/Pamj. 2013.16.102.1450.

Charbonneau, G., Henning, B. M., Low, D. E., Burton, L., and Broukhanski, G. (2006). Triple reassortant H3N2 influenza A viruses, Canada, 2005. *Emerging Infectious Diseases,* 12 (7): 1132–1135.

Eileen, T. and Bruce, J. (2008). Swine Influenza Virus, zoonotic potential and vaccination strategies for the control of avian and swine influenzas. *Journal of Infectious Diseases*, 197: 19 – 24 http://www.socialresearchmethods.net/tutorial/Mugo/tutorial.htm.

Jeong, K., Park, Y.I., Jin w., Han, J.H., Jeong, H.K .,Kim, H.J and Rha, J. (2004). Sero-prevalence of swine influenza in Korea. *Proceeding 18th International pig Veterinary Society Congress*, Hamburg, Germany. P123.

Kirunda, H., Erima, B., Tumushabe, A., Kiconco, J., Tugume T., Mulei, S., Mimbe O., Mworozi, E. Bwogi, J., Luswa, L., Kibuuka, H., Millard, M.

Byaruhanga, A., Ducatez, M.f., Krauss, S., Webby, R.J. Webster, R,G., Wurapa, K., Byarugaba, D.K. and Wabwiremnagen, F. (2014). Prevalence of influenza A viruses in livestock and free-living water fowl in Uganda. *BMC Veterinary Research*, 10:50. http://www.biomedrentral.com /174-6148/10/50.

Markowska-Daniel, I and Stankevicius, A. (2005). Seroprevalence of antibodies against swine influenza virus in pigs of different age. *Bulletin Veterinary Institute of Pulawy*, 49:3-7.

Meseko, C., Olaleye, D., Capua, I. and Cattoli, G. (2013). Swine Influenza in Sub-Saharan Africa – Current Knowledge and Emerging Insights. *Zoonoses and Public Health*, *doi:10.1111/zph.12068*.

Meseko, C.A., Odaibo, G.N. and Olaleye, D.O. (2010): Biosecurity practices and swine influenza exposure risks a case study of a commercial pig farm. Proceedings of the 1st Nigerian International pig summit. Institute of Agricultural Research and Training, Moor Plantation, Ibadan, pp. 162-167.

Mugo, F.W. (2008). Sampling in Research. Accessed from Office International des Epizooties (2010) (World Organization for Animal Health): Chapter 2.8.8. Swine influenza. OIE Terrestrial Manual, 2010.pp1-11.

Office International des Epizooties (2010) (World Organization for Animal Health): Chapter 2.8.8. Swine influenza.OIE Terrestrial Manual, 2010.pp1-11.

Oladipo, E.K Adeniji, J.A. and Fagbami, A.H. (2013). Isolation of influenza A and B viruses from pigs at Bodija Abattoir, Ibadan, Nigeria. *Global Veterinaria*, 11(2): 252-257.

Olsen, C. W., Karasin, A. I., Carman, S., Li, Y., Bastien, N., Ojkic, D., Alves, D., Charbonneau, G., Henning, B. M., Low, D. E., Burton, L., and Broukhanski, G. (2008). Triple reassortant H3N2 influenza A viruses, Canada, 2005. *Emerging Infectious Diseases,* 12 (7): 1132–1135.

Takemae, N. Parchariyanon, S. Ruttanapumma, R. Hromoto, Y., Hayashi, T. and Uchida, Y.S.T. (2011). Swine influenza virus in Thailand. *Virology Journal*, 8: 537-542.

PATHOGENICITY OF *PASTEURELLA MULTOCIDA* STRAINS (SEROTYPES A: 1, 3 AND 4) IN COMMERCIAL CHICKENS AND JAPANESE QUAILS IN JOS, NIGERIA

Dashe, Yakubu Gunya

National Veterinary Research Institute, Akure Zonal Office, Ondo State.

***Correspondence:** yakubudashe@yahoo.co.uk; phone: +234 8035778490

INTRODUCTION

Poultry such as chickens, quails, ostriches offer the quickest supply of animal protein to man, and they provide comparatively faster returns on investment than cattle, small ruminants or pigs (Etukudo and Adegboye, 1983). Japanese quails are highly prolific, they have short generation time and the meat has low cholesterol (Haruna *et al.*, 1997). Quails have been reported to be generally resistant to common diseases that may adversely affect other birds; although they can still be affected by poultry diseases (National Research Council, 1991). Despite the unquantifiable economic gains from this industry, it often suffers disease induced losses due to sustained challenges from environmental factors, viral, bacterial, protozoan, parasitic and fungal infections among others. The epizootic of fowl cholera in the world poses a serious threat to poultry. Fowl cholera has often occurred as a sporadic disease in many parts of the world including Nigeria with grave economic consequences on livestock and humans (Ambali *et al.*, 2003). Fowl cholera is endemic in regions of Asia and Africa where it causes immense economic losses to the poultry industry due to cost of treatment, weight loss and mortality (Townsend and Papadimetrious, 1997). Despite the established role of *P. multocida* as an etiologic agent of fowl cholera in chickens and quails in Jos, Nigeria, there is inadequate information on the clinical, gross pathologic and histopathologic features of the disease caused by *P. multocida* serotypes. This study therefore seeks to document the clinicopathologic features observed in chickens and quails inoculated with *P. multocida* serotypes A: 1, 3 and 4.

Seminar presented 23rd July, 2015 at NVRI Auditorium

MATERIALS AND METHODS

Management and housing of experimental chickens and quails

Thirty 6-week-old (male and female) Japanese quails (*Coturnix coturnix japonica*) and thirty commercial chickens of eighteen weeks old were provided by the National Veterinary Research institute, Vom, for this study. The thirty chickens were divided into groups A, B, C, D and E of 6 chickens each. Groups A, B, C and D were further sub-divided into three sub-groups, with each group consisting of two chickens. One week to the onset of the study, all birds were screened for the presence of *P. multocida* by taking oro-pharyngeal swabs. The chickens were fed with commercial pelleted layer feed and water *ad libitum* before and during the experiment. An experimental pen with adequate floor space ($4x5M^2$) was cleaned, disinfected and used for the study. Chickens were allowed to acclimatize for two weeks prior to the commencement of the pathogenicity study.

Similarly, the thirty Japanese quails were divided into groups as earlier described for the chickens. Birds in each group were housed separately in wire mesh cages with floor space of 35 cm by 35 cm in size in an enclosed house which was cleaned and disinfected weekly. The birds were fed on layer mash and water given *ad libitum*.

Source of Pasteurella multocida

The isolates of *P. multocida* serotypes A: 1, 3 and 4 were recovered by the authors from chickens with clinical cases of fowl cholera in Jos, Nigeria. The isolates were confirmed by biochemical test, Microbact, Polymerase Chain Reaction and Multiplex PCR. They were sorotyped at USDA in USA.

Inoculation of Quails and Chickens

Five groups (six chickens each and six quails each) were used for the pathogenicity test. The first group of chickens (group A) were inoculated with a concentration of 10^8, 10^7 and 10^6 CFU of *P. multocida* serotype A: 1 (contained in 0.1ml of Typtose Soya broth) intramuscularly at the breast muscle. The same concentration of 10^8, 10^7 and 10^6 CFU of A:3 were administered to the second group (group B), while serotype A:4 was given to the third group (C) at the same concentrations, while the 4^{th} group (D) were given vaccine strain at the same concentrations stated above. Appropriate control, i.e., the 5^{th} group (E) was inoculated with 0.1 ml normal saline. The procedure above was carried out for the five groups of Japanese quails. Death occurring within the period of

twenty one days of the experiment was recorded. Findings from clinical, postmortem examination of carcasses and histopathological features were recorded.

Mice Inoculated with Pasteurella multocida

Five groups of six mice each were used for the pathogenicity test. In the first three groups consisting of eighteen apparently healthy 4 week old Swiss-Webster mice were challenged by inoculating intra-peritoneally with 10^8, $10^7$and 10^6 CFU of *P. multocida* serotypes A: 1, 3 and 4 in 0.1 ml Tryptic Soya broth and returned to their cages (floor space of 30 cm^2). Mice were fed with pelleted feed and water *ad libitum* before and during the experiment. The mice were observed for death over 24 h. The fourth group consisting of six mice was inoculated with *P. multocida* vaccine strain obtained from National Veterinary Research Institute, Vom, at the same concentrations above. The fifth group which was the control also consisted of six apparently healthy mice and received 0.1 ml normal saline. All experimental mice were kept under the same environment.

Clinical and pathological examinations

Chickens and quails in all the groups were observed for clinical signs and mortality post inoculation. Postmortem examinations were conducted on chickens and quails that died; and tissue section of the spleen, liver, heart and lungs were prepared for histology. The section of grossly affected organs were fixed in 10% buffered formalin; paraffin embedded tissues were sectioned at 5 microns, stained with haematoxylin and eosin (H &E) and mounted on glass slides and examined under light microscope at x200 and x400 respectively.

Statistical analysis

The entry and sorting of primary data was performed with Microsoft excel, 2010. Descriptive statistical analysis was conducted using statistical package for social sciences SPSS (version 12.01) (2004) and the results were summarized as percentages in tables.

RESULTS

Chickens

Ninety two percent (92%) of the experimental chickens in groups A and B which were inoculated with *P. multocida* serotypes A: 1 and A: 3 manifested clinical signs such as depression, inappetence, ruffled feathers, dyspnoa, sitting on hock within 24 h post inoculation. No clinical sign was noticed in groups (C, D and E). On day five, four carcasses were picked in the group inoculated with *P. multocida* serotypes A: 1 at concentration of 10^8 ,one carcass at 10^7 CFU and three in groups inoculated with *P. multocida* serotype A: 3 at concentrations of 10^8 and 10^7 CFU. No death was recorded in vaccine strain, A: 4 and control groups (Table 1). Lesions observed during postmortem examinations in chickens inoculated with *P. multocida* serotypes A1 and A3 were: prominent keel with congested heart, liver, kidneys and lungs (Table 2). Frothy exudates from the lungs, petechial and ecchymotic haemorrhages were seen on the heart, subserosal haemorrhages (Plate I). lymphocytic, heterophilic and macrophagic cellular infiltration in lungs and heart; multifocal haemorrhages in lungs were observed (Plate II).

Japanese Quails

All Japanese quails in groups A, B, and C became sick within 24 h. Weakness, inapettence; somnolence and sudden death were also observed in the three groups. No clinical signs and mortality were recorded in groups D (vaccine strain) and E (normal saline). Congested heart, liver, lungs, petechial and ecchymotic hemorrhages were noticed on the heart and breast muscles (Table 3). Mortality rates were 100% for concentrations of 10^8 and 10^7 CFU in group A and B, while 0% mortality recorded for 10^6 CFU groups B and C (Table 4). Histopathological findings in this study indicated a moderate to severe lymphocytic, heterophilic and macrophages cellular infiltration in the cardiac muscles of experimental infected Japanese quails (Pate III).

Mice

All the 18 Swiss-Webster mice inoculated with *P. multocida* serotypes: A: 1, A: 3 and A: 4 at concentrations of 10^8, 10^7 and 10^6 CFU were found dead within 24 h post inoculation.

Table 1: Mortality in chickens inoculated intramuscularly with 0.1ml of *Pasteuralla multocida* serotypes A: 1 A: 3 and A: 4

Groups	*Pasteurella multocida* serotype	Conc. of Inoc.	Num. Inoc.	Mortality per day					
				1	2	3	4	5	9
A	A: 1	10^8	2					2/2(100%)	
		10^7	2					2/2(100%)	
		10^6	2					1/2(50%)	
B	A: 3	10^8	2					2/2(100%)	
		10^7	2					1/2(50%)	
		10^6	2					(0%)	
C	A: 4	10^8	2					(0%)	
		10^7	2					(0%)	
		10^6	2					(0%)	
D	Vaccine Strain	10^8	2					(0%)	
		10^7	2					(0%)	
		10^6	2					(0%)	
E	Control (Normal Saline)	-	6					(0%)	

Table 2: Gross lesions in chickens inoculated with *Pasteurella multocida* serotypes A: 1, A: 3 and A: 4

	Pasteurella multocida serotypes		
Gross Lesions	A1	A3	A4
Congested liver	6/6(100%)	6/6(100%)	0/6(0%)
Congested heart	6/6 (100%)	6/6 (100%)	0/6 (0%)
Congested kidneys	6/6 (100%)	6/6(100%)	0/6(0%)
Congested lungs	6/6 (100%)	6/6 (100%)	0/6(0%)
Frothy exudates from lung	3/6 (50%)	0/6 (0%)	0/6 (0%)
Haemorrhages on the heart	6/6 (100%)	6/6 (100%)	0/6(0%)
Fatty degeneration	1/6 (16.7%)	0/6(0%)	0/6 (0%)
Egg yolk peritonitis	3/6 (50%)	3/6 (50%)	0/6(0%)
Prominent keel	6/6 (100%)	6/6 (100%)	0/6 (0%)

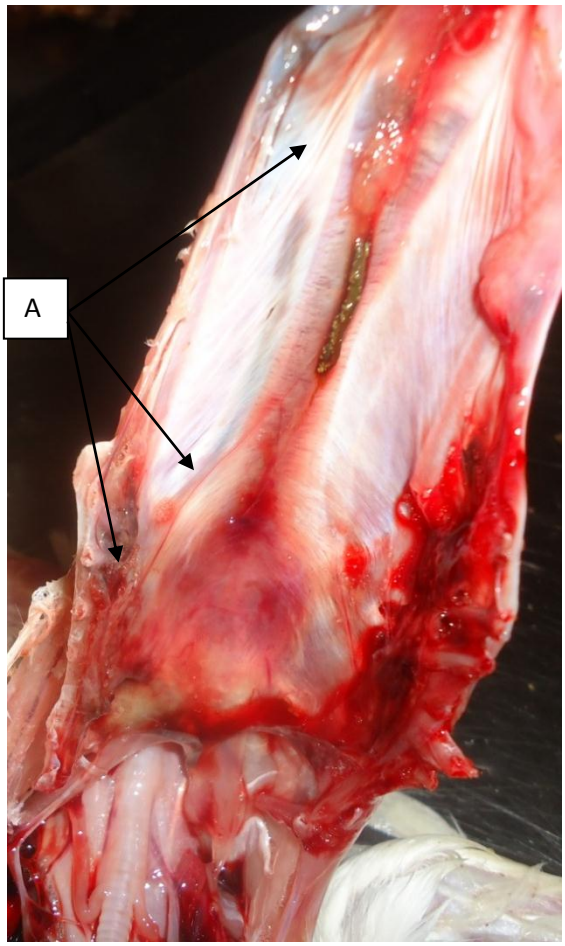
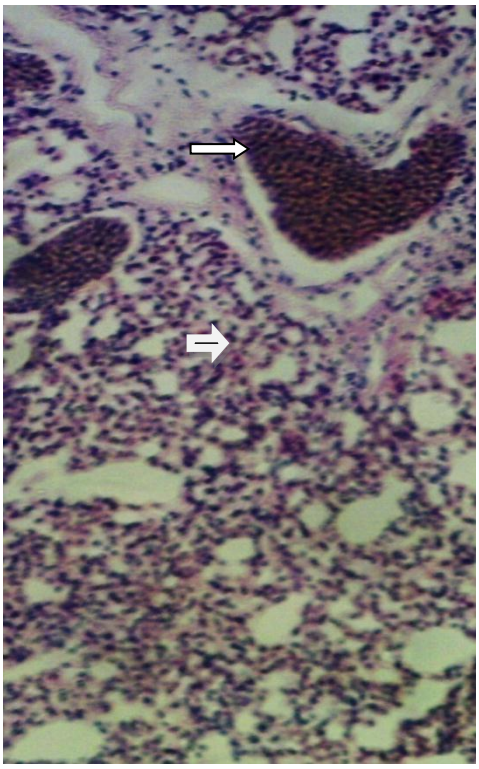
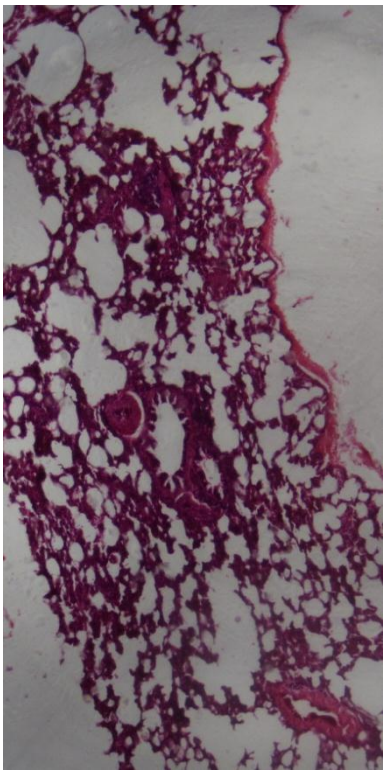


Plate I: A - Haemorrhages (arrowhead) on the pectoral muscle of chicken inoculated with *Pasteurella multocida* Serotypes A: 1.



a.



b.

Plate II a: Photomicrograph of a lung from a chicken infected with *Pasteurella multocida* A: $3=10^8$. Note the congested blood vessels (small arrow), the areas with obliterated alveoli and infiltrated with inflammatory cells (big arrow) H and E Stain. X 200. b: normal lung of a chicken at X 400.

Table 3: Gross lesions in Japanese quail Inoculated with *Pasteurella multocida* Serotypes A: 1, A: 3 and A: 4

Gross Lesions	A1	A3	A4
Congested heart	6/6 (100%)	6/6 (100%)	3/6 (50%)
Congested liver	6/6 (100%)	6/6 (100%)	6/6 (100%)
Congested lungs	3/6 (50%)	3/6 (50%)	3/6 (50%)
Haemorrhages on the heart	6/6 (100%)	6/6 (100%)	0/6 (0%)
Hemorr of pectoral muscles	3/6 (50%)	3/6 (50%)	0/6(0%)

Table 4: Mortality in Japanese quails inoculated intramuscularly

with 0.1ml of *Pasteurella multocida* serotypes A: 1 A: 3 and A:4

Group	*Pasteurella multocida* serotype	Conc. of inocu	Numb. Inocul.	Day mortality
				Day 7
A	A: 1	10^8	2	2/2(100%)
		10^7	2	2/2(100%)
		10^6	2	1/2(50%)
B	A: 3	10^8	2	2/2(100%)
		10^7	2	2/2(100%)
		10^6	2	(0%)
C	A: 4	10^8	2	2/2(100%)
		10^7	2	(0%)
		10^6	2	(0%)
D	Vaccine Strain	10^8	2	(0%)
		10^7	2	(0%)
		10^6	2	(0%)
E	Control Normal Saline	-	6	(0%)

Key
Inocul – Inoculation
Inocu – Inoculum
Conc = Concerntration
Numb = Number

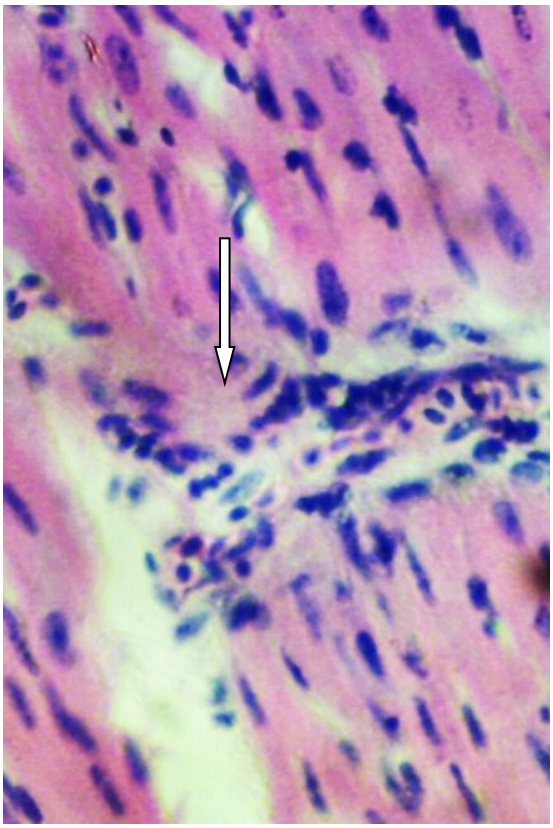


Plate III: Photomicrograph of cardiac muscle of a Japanese quail inoculated with *P. multocida* A: 4= 10^8. Note the infiltrated inflammatory cells in an area of myocardial necrosis (arrow). H and E Stain X 400.

DISCUSSION

Pasteurella multocida is one of the bacterial agents responsible for significant economic losses in poultry industry worldwide. The report of this study indicates that only serotypes A: 1 and 3 caused clinical disease in chickens with high morbidity and mortality; while Japanese quails were susceptible to *P. multocida* serotypes A: 1, 3, 4. Interestingly, these serotypes also demonstrated various degree of gross and histopathological lesions in the experimental chickens and Japanese quails. In a similar study conducted in India by Kumar *et al.* (2004), they opined that majority of *P. multocida* belonging to serotypes A:

1, 3 and 4 were associated with fowl cholera in chickens. The findings in this study differ slightly from that of Kumar and others in that serotype A: 4 in this study did not cause mortality in chickens. Another report by Elfak *et al.* (2002) reported the involvement of *P. multocida* serotypes 1, 3, 4 and 5 in an outbreak of fowl cholera in ostriches. The grave implication of these reports is that the production of universal fowl cholera vaccine would be difficult considering the fact that *P. multocida* serotypes vary from one country to the other and immunity is known to be serotype specific.

The gross lesions observed at postmortem in the experimentally challenged chickens support the findings of Visut *et al.* (2010). The result of this pathogenicity study indicates that all the experimental chickens inoculated with serotypes A: 1 and A: 3 showed clinical signs of fowl cholera similar to those reported by Rimler and Glisson (1997). This buttressed the fact that adult chickens above 16 weeks of age are susceptible to fowl cholera. The widespread pathological lesions that were observed in all the organs of quails were indications of bacterial septicemia; this could possibly explain the profound debilitation observed in birds suffering from acute form of fowl cholera. Similar findings were previously reported by Odugbo *et al.* (2004). Akpavi *et al.* (2011) reported 92% mortality in Japanese quails inoculated with *P. multocida* serotype A: 4; their figure was rather high compared to the 33.3% (group C which was inoculated *P. multocida* serotype A: 4) in this study. The difference could be attributed to depression in immunity which led to high susceptibility in the present study. Myint and Carter (1988) also reported 60% mortality in a natural outbreak of fowl cholera in quails in Burma. In this study, mortality in the Japanese quails was first noticed only on day seven post inoculation. This report is at variance with the findings Akpavi *et al.* (2011) who reported that mortality was first noticed one day post inoculation and lasted five days with highest mortality rate on day one post inoculation. High mortality was recorded in groups of quails inoculated with *P. multocida* serotypes A: 1, 3 and 4 at concentration 10^8, this finding showed that there is a correlation between the severity of fowl cholera and high concentration of *P. multocida* inoculum. This report differs from the findings Akpavi *et al.* (2011) who reported that there was no correlation between the dose of inoculum and mortality rate.

All field isolates of *P. multocida* recovered in this study were found to be pathogenic to Swiss-Webster mice. They exhibited high pathogenicity by causing 100% mortality in mice within 24 h post inoculation.

The findings of this study have revealed that chickens and Japanese quails are highly susceptible to infections with *P. multocida* serotypes A: 1, and 3 and in

addition, Japanese quails were also susceptible to serotype A: 4. It is therefore recommended that Japanese quails should be vaccinated against fowl cholera and the current fowl cholera vaccine by National Veterinary Research Institute should be revalidated by producing a polyvalent vaccine consisting of *P. multocida* serotypes A: I, 3, and 4 in order to protect birds against *P. multocida* infections.

REFERENCES

Akpavi, V., Abdu, P. A., Mamman, P. H. and Sa'idu, L. (2011). Clinicopathological features in Japanese quails (*Coturnix coturnix japonica*) infected with *Pasteurella multocida multocida*. *Sahel Journal of Veterinary Science*, 19: 15-20.

Ambali, A. G., Abubakar, N. B. and James, T. E. (2003). An assessment of poultry health problems in Maiduguri, Borno State, Nigeria. *Tropical Veterinarian,* 21: 138-145.

Elfak, M. G., Abbas, B., Mahmoud, M. O., Haroun, E. M. and Abdel-Magied, E. M. (2002). Septicaemic pasteurellosis in ostriches (*Stricthio camelus*) in Central Saudi Arabia. *The Veterinary Journal*, 163: 218-221.

Etukudo, B. O. and Adegboye, D. S. (1983). Studies of chronic respiratory diseases of poultry in Nigeria. Bacteriological examination of clinically diagnosed cases. *Nigerian Veterinary Journal*, 12: 66-69.

Haruna, E. S., Musa, U., Okewole, P. A., Shamaki, D., Lombin, L. H., Molokwu, J., Edache, J.A. and Karsin, P. D. (1997). Protein requirement of quail chicks in Plateau State, Nigeria.*Nig. Vet. Journal,* 18:108-113.

Kumar, A. A., Shivachandra, S. B., Biswas, A., Singh, V. P., Vijendra, P. and Srivastava, S. K. (2004). Prevalent serotypes of *Pasteurella multocida* isolated from different animals and avian species in India. *Veterinary Research Communication*, 28: 657- 667.

Myint, A. and Carter, G. R. (1988). Fowl cholera in quails in Burma. *Tropical Asian Health Produc.* 20:35-36.

National Research Council (NRC). (1991). *The quail bird*. Board for Science and Technology for International Development, Academic Press, Washington, D. C. Pp. 147-155

Odugbo. M. O., Muhammad, M., Musa, U., Suleiman, A. B., Ekundayo, S. O. and Ogunjumo, S. O. (2004). Pasteurellosis in Japanese quail (*Coturnix coturnix japonica*) caused by *Pasteurella multocida multocida* A: 4. *Veterinary Record,* 155: 90-91.

Rimler, R. B. and Glisson, J. R. (1997). Fowl cholera. In: Calnek, B. W., Barnes, H. J., Beard, E. W., McDougald, L. R. and Saif, Y. M. (Eds), *Diseases of Poultry,* 10th edition, Iowa State University Press, Ames, Iowa, U.S.A, Pp. 143-159.

Statistical Package For Social Science (SPSS), (2004), Version 12.01. Chicago Incorporated, United States of America.

Townsend, K. M. and Papadimetrious, J. M. (1997). REP-PCR analysis of *Pasteurella multocida* isolates that cause haemorrhagic septicaemia. *Research in Veterinary Science,* 63: 151- 155.

Visut, R., Piyarat, C. and Niwat, C. (2010). Comparison of efficacy of enrofloxacin against *Escherichia coli* or *Pasteurella multocida* infection in chickens. *Thai Journal of Veterinary Medicine*, 40: 297-301.

ISOLATION OF *BRUCELLA ABORTUS* BIOTYPE 3 FROM CATTLE IN KACHIA GRAZING RESERVE AND JOS PLATEAU AND THEIR IDENTIFICATION BY BRUCE-LADDER MULTIPLEX PCR

***Bertu[1], W. J; Kwaga[2], J. K. P; Kabir[2], J; Ocholi[3], R. A and I. Moriyon[4]**

1. Brucellosis Research Laboratory, Bacterial Research Division, National Veterinary Research Institute, Vom, Nigeria.

2. Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria

3. Quality Control Division, National Veterinary Research Institute, Vom, Nigeria.

4. Department of Microbiology and Parasitology, Medical School and Institute for Tropical Health, Universidad de Navarra, Pamplona, Spain.

Correspondence: wilchris2003@yahoo.com

INTRODUCTION

Brucellosis is caused by slow-growing, small, gram negative, cocobacilli, facultative intracellular bacteria belonging to the genus *Brucella*. Six species were earlier recognized which include *Brucella abortus* (cattle), *Brucella melitensis* (goats and sheep), *Brucella suis* (pigs), *Brucella ovis* (sheep), *Brucella canis* (dog), *Brucella neotomae* (desert wood rat) (Osterman and Moriyon, 2006). Much later, two species were reported from marine mammals namely, *Brucella ceti* (porpoises and dolphins) and *Brucella pennipedialis* (seals) (Foster *et al.*, 2007). *Brucella microti* was later isolated from the common vole (*Microtus arvalis*) and *Brucella inopinata* from human breast implant (Scholz *et al.*, 2010; Nymo *et al.*, 2011).

Members of the genus *Brucella* represent some of the world's major zoonotic pathogens responsible for enormous economic losses and considerable human morbidity (Pappas *et al.,* 2006). Brucellosis remains a major bacterial zoonotic disease of global importance (Cutler *et al.*, 2005). While *Brucella* is sometimes

cited as a re-emerging pathogen, often reflecting socioeconomic changes in particular locations, the organism has been recognised as a major scourge of mankind since its first isolation from humans (Bruce, 1887) cited by Alton *et al.* (1988). It is also regarded as a major problem among ruminants in sub-Saharan Africa and its epidemiology and impact has been reported in several countries (Mcdermott and Arimi, 2002; Bronsvoort *et al.,* 2009).

Key to understanding of brucellosis epidemiology is identification of circulating *Brucella* species (and biovars). This is because antibodies are not species specific. There are various reports on cultural isolation of *Brucella* organisms from Nigerian livestock over the years.

Although, isolation of the organism remains the most reliable and confirmatory diagnosis of brucellosis (OIE, 2009; FAO, 2003), it is usually laborious, time consuming and sometimes very hazardous (Lopez-Goni *et al.*, 2008; OIE, 2009).

The advent of molecular methods, especially polymerase chain reaction (PCR) technique has enhanced disease diagnostic capacities to a point that identification of causative agents is less obscure (Ron-Roman *et al.*, 2012). One of these methods is a multiplex conventional PCR called Bruce-Ladder (Garcia-Yoldi *et al.,* 2006; Lopez-Goni *et al.*, 2008). The Bruce-ladder multiplex PCR is robust, performs very well and can differentiate all known *Brucella* species and the vaccine strains and is as sensitive as classical culture-based techniques (Lopez-Goni *et al.*, 2008).

The Kachia Grazing reserve and the Jos Plateau are home to semi-sedentary and nomadic Fulani herdsmen that practice extensive animal husbandry with free movement of the animals within and outside their locations. This life style could encourage the spread of brucellosis among cattle and herdsmen in the areas. The aim of this study was to isolate *Brucella* organisms from cattle in the two study areas and carry out classical biotyping and molecular characterization of the isolates using Bruce-ladder multiplex PCR.

MATERIALS AND METHODS

Study Areas

Kachia Grazing reserve (KGR) in Kaduna state is located between Latitude 10.10112-10.29477^{0}N and Longitude 8.00708-8.15154^{0}E. It is a wide span of

flat lowland area covering about 33, 411 hectares covering three LGAs in the present Kaduna state, namely Kachia, Kajuru and Zangon Kataf. The livestock population in the Grazing Reserve was made up of 41,234 cattle, 10,161 sheep, 4, 828 goats. The *Jos Plateau* study area comprises villages in Bokkos, Mangu and Pankshin LGAs of Plateau State. It comprises 10 selected villages with several households owning a total of 10,264 cattle.

Sampling Design

A purposive sampling technique was used. Samples were collected only from cattle with history of abortion, repeat breeding and those with hygroma.

Sample Collection and Handling

Fifty five (55) vaginal swabs, 70 milk samples, 2 hygroma fluids and 1 placenta were collected from cattle in selected herds in KGR while 63 vaginal swabs, 36 milk samples and 2 hygroma fluids were collected from cattle on the Jos Plateau. Milk samples and hygroma fluids were collected into sterile 10 ml sample tubes and 10 ml syringes respectively, all containing tryptone broth (transport medium) and properly labelled. Vaginal swabs were collected using cotton swabs sticks in which 2ml of tryptone broth was added by the manufacturers. The samples were transported on ice in cold boxes to the Brucellosis Research Laboratory at the National Veterinary Research Institute, Vom for culture.

Culturing of samples and biochemical tests

All samples for isolation were cultured immediately on arrival to the laboratory on serum dextrose agar (SDA) or trypticase soy agar (TSA) (*Brucella* selective media) for isolation of *Brucella* organisms as described by Alton *et al.* (1988). The inoculated plates were incubated at 37^0C for 3-7 days in the presence of 5-10% CO_2 atmosphere.

Isolates that appeared typical of *Brucella* organisms under the microscope were further subjected to classical biotyping tests as described by Alton *et al.* (1988). Isolates were then lyophilized and stored at -20^0C for further work.

Classical Biotyping

All the sixteen *Brucella* isolates were identified using *Staphylococcus aureus* Cowan 1test. They were then subjected to classical biotyping tests as described by Alton *et al.* (1988).

Bruce-Ladder Multiplex PCR

Extraction of genomic DNA

Genomic DNA was extracted from pure *Brucella* cultures using a standard commercial microbial DNA extraction kit (QIAamp DNA Mini Kit, Homburg, Germany) according to the manufacturer's instructions. DNA was then extracted by a standard protocol with phenol-chloroform-isoamyl alcohol, precipitated with isopropanol, washed with ethanol. The DNA pellet was re-dissolved in 50-100µl of sterile distilled water for PCR.

Preparation of primer cocktail

0.5µl of the 16 primers (8 pairs) was aliquoted into a pre-labelled 1.5ml thin walled microfuge tube. A total of 8 µl of primer cocktail was required for each isolate. This was prepared for 25 isolates, giving a total volume of 200 µl of primer cocktail which was briefly vortexed to ensure homogeneity.

Preparation of Master Mix

Reagents	Final conc.	1rxn	x 25rxn
PCR buffer 10 X	1x	2.5µl	62.5
dNTPs Mix (2mM)	400mM each one	5.0µl	125.0
$MgCl_2$ (50mM)	3.0mM	1.5µl	37.5
Bruce-ladder eight primer cocktail (12.5)	6.25pmol each one	7.6µl	190.0
H_2O (PCR grade)	-	7.1µl	177.5
DNA Polymerase	1.5U	0.3µl	7.5
Total Volume		24.0µl	600.0µl

24 µl was pipetted into 25 pre-laballed 0.2 ml thin walled microfuge tubes. 1.0 µl of template DNA was added to the 24 µl PCR reaction mixture, giving a total volume of 25 µl for each sample.

PCR Cycling Conditions

PCR reaction mixture was placed in a thermocycler and set for amplification. The initial denaturation temperature was 95 ^{0}C for 7 minutes, template denaturation was 95 ^{0}C for 35 seconds and primer annealing at 64 ^{0}C for 45 seconds. This was followed by primer extension at 72 ^{0}C for 3 minutes, final extension at 72 ^{0}C for 6 minutes for a total of 25 cycles and cooled at 4^{0}C.

Gel Electrophoresis and Analysis

After PCR amplification, 2 µl of PCR product and 8 µl of bromophenol blue (loading buffer) were loaded into wells in 1.5% agarose gel in TBE in a cuvette flooded with TBE slightly covering the gel.

100-bp DNA ladder or 1kb plus DNA ladder (Invitrogen LTD) (www.invitrogen.com) was used as molecular marker.

The gel was stained with 7% SYBR Safe DNA Gel Stain (www.lifetechnologies.com). The electrophoresis equipment was set to run at 130V for 50 minutes. The gel was then visualised under UV light in the computerized gel documentation equipment.

RESULTS

Three *Brucella abortus* isolates were obtained from Kachia Grazing Reserve while four (4) *Brucella abortus* were isolated from Jos Plateau. The isolates grew after 3 days incubation at 37^{0}C in the presence of 10% CO_2 and without CO_2 respectively.

The results of Bruce-ladder multiplex PCR for all the *Brucella* isolates obtained in this study are presented (Figures 1).

The amplicons of the isolates were compared with those of the reference *Brucella* strains. The Bruce-ladder multiplex PCR from DNA from the sixteen *Brucella abortus* amplified five fragment sizes of 1,682bp, 794bp, 587bp, 450bp and 152bp which were consistent with amplicons amplified by DNA of *Brucella*

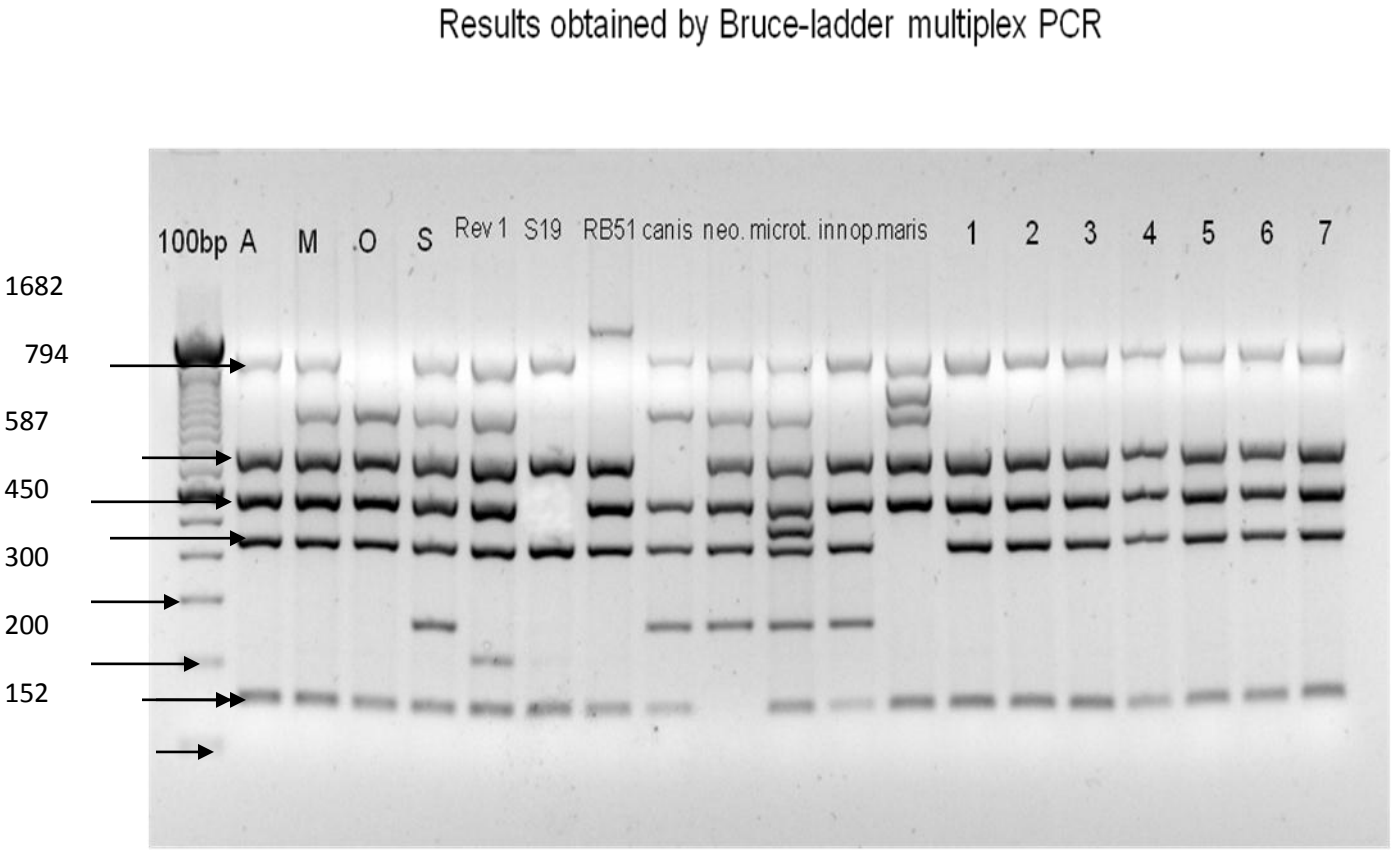
abortus S19 reference strain used as control. All the 16 *Brucella abortus* isolates were therefore confirmed as *Brucella abortus* by the Bruce-ladder multiplex PCR.

Table 1: Biotyping of *Brucella abortus* isolates from this study based on their growth on agar plates containing various concentrations of thionin and basic fuchsin dyes

Isolates / Ref. Strains	Growth on Thionin dye			Growth on basic Fuchsin dye		*Brucella* Species and Biovars
	10µg/ml	20µg/ml	40µg/ml	10µg/ml	20µg/ml	
S19*	-	-	-	+	+	
1330*	+	+	+	-		
16M*	+	+	-	+	+	
Ovis63/290*	+	+	-	-	-	
Rev.1*	+	+	-	-	-	
KGR1**	+	-	-	+	+	*B.abortus 3*
KGR2**	+	-	-	+	+	*B.abortus 3*
KGR3**	+	-	-	+	+	*B.abortus 3*
PLM1**	+	-	-	+	+	*B.abortus 3*
PLB2**	+	-	-	+	+	*B.abortus*

						3

Key: * *Brucella* control strains
** *Brucella* isolates from this study



Molecular marker (100bp); lane 1, *B. abortus* 2308; lane 2, *B.melitensis* 16M; lane 3, *B. ovis;* lane 4, *B. suis* 1330*;* lane 5, *B. melitensis* Rev1vaccine 6, *B. abortus* S19 vaccine; lane 7, *B. abortus* RB51 vaccine; lane 8, *B. canis*; lane 9, *B. neotomae*; lane 10, *B. microti*; lane 11, *B. inopinata;* lane 12, *B. ceti*; lane 13 (1), MBL; lane 14(2), KGR2; lane15(3), PLM1; lane 16(4), PLB2; lane 17(5), KGR2;

Figure 1: Gel photograph of Bruce-ladder multiplex PCR for *Brucella* isolates 1-7 and the 12 *Brucella* reference control strains

DISCUSSION

Since brucellosis was first reported in Nigeria in 1927 (Banerjee and Bhatty, 1970), only five bacteriological studies have been undertaken in cattle in various locations and *Brucella abortus* isolated (Ducrotoy *et al.*, 2014). There is concern that brucellosis could emerge as a result of the settling of previously migratory herds or from increased contacts between infected nomadic herds and susceptible intensive commercial or settled semi-intensive herds (Ducrotoy *et al.*, 2014). This concern has been confirmed by the successful isolation of *Brucella abortus* in both KGR and on the Jos Plateau, indicating that brucellosis is endemic in the study areas. This is very significant and has far reaching implications as the infected cows may spread the infection to other cattle within the herds as well as sheep and goats which are usually reared along with cattle as the organisms are shed on pasture. Infection could also be spread from infected herds to non-infected herds through contact and through loaning of heifers and bulls commonly practiced by herdsmen.

The isolation of *Brucella abortus* from milk in both KGR and Jos Plateau is of great public health significance because *Brucella* organisms are usually shed through the milk of infected cows which is consumed raw or as fermented milk. The most common means of transmission of brucellosis from animals to humans is through the consumption of unpasteurized or raw milk and milk products (Kang'ethe *et al.*, 2000, Capasso, 2002).

The isolation of *Brucella abortus* from the vaginal swab is also of great public health significance. This is because herdsmen are in the habit of using their bare hands to assist cows to ensure successful parturition in cases of dystocia or to pull out placenta in cases of retained placenta. These habits and practices expose them to high risk of contracting brucellosis through direct contact with the organisms.

There was higher rate of isolation of *Brucella* organisms from hygroma fluids in this study compared to other samples because hygroma is unlikely to get contaminated compared to other samples. This finding is consistent with reports of previous investigators in Nigeria where a majority of isolates were from hygroma fluid and only a few from other samples such as milk, vaginal swabs, aborted foetuses and placentae (Eze, 1978; Bale and Kumi-Diaka, 1981; Ocholi *et al.*, 2004b). The finding also agrees with reports of other authors that found that although *Brucella* could be isolated from other samples and tissues, hygroma is the most valuable in terms of success rate of isolation in nomadic

and semi-nomadic cattle herds in Africa (Corbel, 2006; Godfroid *et al.*, 2010; OIE, 2009).

Based on classical biotyping, the isolates were all identified as *Brucella abortus* biotype 3. This finding differs from those of previous investigators (Eze, 1978; Ocholi *et al.*, 2004a, b; Ocholi *et al.*, 2005) in which *Brucella abortus* biotype 1 was reported as the prevailing biotype in Nigeria. Differences between biotypes 1 and 3 are not very apparent and can only be distinguished based on their sensitivity to thionin dye (Banai and Corbel, 2010). While *Brucella abortus* S19 Biotype 1 did not grow in presence of thionin at all three concentrations of 10µg/ml, 20µg/ml and 40µg/ml, all the isolates tested in this study grew in the presence of thionin only at 10µg/ml which is a typical behaviour of *Brucella abortus* biotype 3. This confirmed all the isolates as *Brucella abortus* biotype 3. The Bruce-ladder multiplex PCR successfully identified all phenotypically characterized *Brucella abortus* isolates to species level as *Brucella abortus.* To our knowledge, this is the first molecular technique used to identify *Brucella* to species level in Nigeria.

The present study in which Nigerian isolates of *Brucella abortus* were identified by Bruce-ladder multiplex PCR has included Nigeria among countries from where *Brucella* strains have been successfully subjected to molecular identification by PCR. These findings have established the endemicity of *Brucella* infection due to *Brucella abortus* biotype 3 in KGR and on the Jos Plateau. The study provides useful data for the establishment of appropriate brucellosis intervention and control measures in the study areas. Phylogenetic studies of the isolates are recommended.

CONCLUSION

The successful isolation of *Brucella* in KGR and Jos Plateau shows that brucellosis is prevalent in the areas and *Brucella abortus* is the prevailing *Brucella* species. All the species from the study areas were *Brucella abortus* biotype 3. Molecular characterization by Bruce-ladder Multiplex PCR of all the isolates from the study areas showed that they were *Brucella abortus.*

ACKNOWLEDGEMENT

This work was supported by a grant from European Union Framework 7, Integrated Control of Neglected Zoonoses (ICONZ) project. We thank the Executive Director, NVRI, Vom for sponsoring this study. We also thank the staff of *Brucella* Research Laboratory and field work team, NVRI Vom, staff of

Department of Microbiology and Parasitology, Medical School and Institute for Tropical Health, Universidad de Navarra, Pamplona, Spain and staff of Centro Investigacion Technologia Agroalimentaria, (CITA) Laboratory, Zaragoza, Spain, for technical assistance.

REFERENCES

Alton G. G., Jones .L. M., Angus R. D., and Verger .J. M. (1988). Techniques for the Brucellosis Laboratory, *Institute National de la Vacherche Agronomique, (INRA) Paris, 1st Edition, pp. 63-129.*

Bale, J. O. O., and Kumi-Diaka, J. (1981). Serological and bacteriological study of bovine brucellae from livestock investigation and breeding centers in Nigeria. *British Veterinary Journal, 137: 256-261.*

Banai, M and Corbel, M. (2010). Taxonomy of *Brucella, Open Access Veterinary Science Journal, 4, 85-101.*

Banerjee AK, Bhatty MA (1970). A survey of bovine brucellosis in northern Nigeria (a preliminary communication). *Bulletin of Epizootic Diseases in Africa, 18, 333–338.*

Bronsvoort, B. M., Koterwas, B., Land, F., Handel, I. G., Tucker, J., Morgan, K. l., Tanya, V. N., Abdoel, T. H., and Smits, H. L. (2009). Comparison of a flow assay for brucellosis antibodies with the reference cELISA test in West African *Bos indicus, PLoS one, 4, 5221*

Bruce, D. (1887). Note on the discovery of a microorganism in Malta fever. *Practitioner, 39, 161.*

Capasso, L. (2002). Bacteria in two-millennia-old cheese, and related epizoonoses in Roman populations*, Journal of Infections 45, 122–127*

Corbel, M. J. (2006) Brucellosis in humans and animals, *WHO Manual, pp. 1-21*

Cutler, S.J., Whatmore, A.M., and Commander, N.J. (2005) Brucellosis –New aspect of an old disease, *Journal of applied Microbiology, 98: 1270-1281.*

Ducrotoy, M. J., Bertu, W. J., Ocholi, R. A., Gusi, A. M., Ward Bryssinckx, W., Welburn, S., Moriyon., I. (2014). Brucellosis as an emerging threat in developing economies: Lessons from Nigeria. *PLOS Neglected Tropical Diseases, 8: 1-18.*

Eze, E.N. (1978). Isolation of *Brucellae* from the Nigeria livestock and the typing of such isolates. *Bulletin of Animal Health Production Africa, 26: 29-36.*

FAO (2003) Guidelines for coordinated human and animal brucellosis surveillance, *FAO animal production and Health, paper No.156, Rome p.56.*

Foster, G., Osterman, B. S., Godfroid, J., Jacgues, I., and Cloeckaert, A. (2007) *Brucella ceti* sp.nov and *Brucella pinnipedialis* sp.nov.for *Brucella* strains with cetaceans and seals as their preferential hosts. *International Journal of Systemic and Evolutionary Microbiology, 57; 2688-2693*

Garcia-Yoldi, D., Marin, C. M., de Miguel, M. J., Munoz, P. M., Vizmanos, J. L., Lopez-Goni,I. (2006). Multiplex PCR for the identification and differentiation of all *Brucella* species and the vaccine strains *Brucella abortus* S19 and RB51 and *Brucella melitensis* Rev.1. *Cinical Chemistry, 52, 779-781.*

Godfroid, J., Nielsen, K., Saegerman, C. (2010). Diagnosis of brucellosis in livestock and wildlife, *Croatian Medical Journal, 51, 296-305*

Kang'ethe, E. K., Arimi, S. M., Omore, A. O., Mcdermit, J. J., Nduhiu, J. G., Macharia, J. K., Githua, A. (2000). Prevalence of *Brucella* antibodies in small holder dairy farms in Kenya, *A paper prepared for oral presentation at the 3rd All African Conference on Animal Agriculture,proceedings, pp.6-9.*

Lo´ pez-Gon˜ i, I., Garcı´a-Yoldi, D., Marı´n, C. M., de Miguel, M. J., Mun˜oz, P. M., Blasco, J. M., Jacques, I., Grayon, M., Cloeckaert, A., Ferreira, A. C., Cardoso, R., Corre^a deSa´, M. I., Walravens, K., Albert, D., Garin-Bastuji, B. (2008). Evaluation of a multiplex PCR assay (Bruce-ladder) for molecular typing of all *Brucella* species, including the vaccine strains, *Journal of Clinical Microbiology, 46: 3484–3487*

McDermott, J. J, Arimi, S. M (2002). Brucellosis in sub-Saharan Africa:

epidemiology, control and impact. *Veterinary Microbiology, 90, 111–134.*

Nymo, I. H; Tryland, M; Godfroid, J. (2011). A review of *Brucella* infection in marine mammals, with special emphasis on *Brucella pinnipedialis* in hooded seal (*Cystophora cristata*), *Veterinary Research, 42, 93*

Ocholi, R.A; Kwaga, J. K. P; Ajogi, I; Bale, J.O. (2005). Abortion due to *Brucella abortus* in sheep in Nigeria, *Revieu Scientific Technical Office Internationale Epizootics, 24 (3), 97*3-979

Ocholi, R. A; Kwaga, J. K. P; Ajogi, I; Bertu, W. J., and Okpara, J. (2004a). Carpal bursitis associated with *Brucella abortus* in a horse in Nigeria, *Veterinary Record, 155:566-567*.

Ocholi, R.A; Kwaga J. K. P; Ajogi, I., and Bale, J. O. O. (2004b). Phenotypic characterization of *Brucella* strains isolated from Livestock in Nigeria. *Veterinary Microbiology 103, 47-53.*

OIE, (2009). World Organization for Animal Health, Chapter 2.4.3.Bovine Brucellosis, *OIE Manual of Diagnostic tests and vaccins for Terrestrial Animals.1-35*

Osterman, B; Moriyon, I. (2006). International committee on systematic of prokaryotes, Subcommittee on the taxonomy of *Brucella.*Report of the September, 2003, Pamplona, Spain, *International Journal of Systemic and Evolutionary Microbiology, 56, 1173-1175*.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., Tsianos, E.V. (2006). The new global map of human brucellosis, *Lancet Infectious Disease, 6, 91–99*

Ron-Roman, J; Saegerman, C; Minda-Aluisa, E; Benitz-Ortiz, J; Brandt, J; Douce, R (2012)First report of orchitis in man caused by Brucella abortus biovar 1 in Ecuador. American Journal of Tropical Medicine and Hygiene, 87:524-528

Scholz, H. C; Nockler, K; Gollner, C; Bahn, P; Vergnaud, G; Tomaso, H; Al Dahouk, S; Kampfer, P; Cloeckaert, A; Maquart, M; Zygmunt, M.S; Whatmore, A; Pfeffer, M; Huber, B; Busse, H. J; De, B.K. (2010). *Brucella*

inopinata sp.nov.isolated from a breast implant infection. *International Journal of Systemic and Evolutionary Microbiology, 60, 801-808.*

Zinsstag, J., Schelling, E., Solera., Blasco, J. M. and I. Moriyón, I. (2011). Brucelosis, pp. 54–62. *In* S. R. Palmer, L. Soulsby, P. R. Torgeson, and D. G. Brown (eds.), Handbook of Zoonoses, *2nd ed. Oxford University Press, Oxford & New York.*

ANTIBODY ENGINEERING: SINGLE DOMAIN ANTIBODY (NANOBODY) AS A RESEARCH TOOL

Obishakin E. T.
Applied Biotechnology Division, National Veterinary Research Institute, Vom, Jos, Plateau State

INTRODUCTION

The structure of conventional immunoglobulin-G (IgG) is composed of highly conserved two identical heavy (H) chains and two identical light (*L*) chains that make up the antigen-binding site. The heavy chain consists of four domains: the variable N-terminal (*VH*), conserved CH1, CH2 and CH3 domains. The light chain consists of two domains; the variable light (*VL*) and the constant light (*CL*) domain. The variable light and the variable heavy chains make up the antigen recognizing fragment (Figure 1 A) (Muyldermans, 2013). Camelids (llamas, camels, and alpacas) and sharks produce heavy-chain antibodies (*HCAbs*) which are devoid of light chains, lacks CH1 domain and are capable of antigen recognition. The antigen-binding fragments comprise of a single domain, and the product of the recombinant expression of this single domain is referred to as nanobody (Figure 1B). In camels, 50-80% of immunoglobulins are devoid of light chains. The antigen-binding part of the nanobody can be produced by recombinant expression in bacteria cells (Figure 1 C). Nanobodies are used in a wide variety of applications because of their small size (15 kilodaltons), high solubility, thermal stability and good tissue penetration. They can be stored for months at 4°C for longer periods at -20°C. Some nanobodies have been shown to resist temperatures above 90°C (Van der Linden *et al.*, 1999).

There have been several attempts to incorporate nanobodies in the place of conventional antibodies in research, diagnosis, therapy and industry. Nanobodies have been generated for the purpose of experimental therapeutic applications against different viruses: HIV, hepatitis B virus, influenza virus, respiratory syncytial virus, rabies virus, FMDV, poliovirus, rotavirus, and porcine endogenous retroviruses. Because of their molecular weight of 15,000 which is smaller than the renal clearance cut off of 50,000, they are eliminated from the blood rapidly making them suitable for *in vivo*-imaging (Vaneycken *et al.,* 2011).

Recently, the investigation of nanobodies for use in therapeutic, diagnostic, trypanolytic, imaging capacities in trypanosome research have been shown.

Ability of VHH to bind into clefts or pockets is greatly enhanced based on the compact nature and its convex paratope. This feature has been greatly exploited as nanobodies are able to bind to the cryptic epitopes of the VSG of trypanosomes that are not exposed to large molecules of convectional antibodies.

MATERIALS AND METHODS

Overview of generation of recombinant antigen specific nanobody

To generate recombinant antigen specific nanobodies, llamas, dromedaries, camels and alpacas are first immunized for 5–7 weeks with 0.5-1mg of proteinaceous antigens with 0.1-0.2 mg of antigen per week. This will allow for the affinity maturation of antibodies and raise the titer of B cell producing antigen specific HCAbs. Freund's adjuvant or Gerbu adjuvants can be used for immunization. Four days after the last immunization, 50-100mls of blood is collected from the camelid. Using lymphocyte isolation methods, lymphocytes are isolated from the blood sample and mRNA is further isolated from the lymphocytes (Deckers *et al.*, 2009). From the total mRNA, a first strand cDNA is produced. The gene fragments encoding the variable domain until the CH2 domain are amplified from the cDNA with specific primers. This yields two separate bands 900 kb (VH– CH1–CH2 exons) and 600kb (VHH–CH2) without CH1 exon. The 600 kb fragment is excised from 1% agarose gel and purified. Furthermore, VHH fragment is amplified from the VHH-CH2 fragment with nested primers encompassing framework-1 and framework-4 of VHH. The VHH fragment is first restricted alongside an expression vector. It is subsequently ligated into expression vectors which could be with or without a detection or purification tag e.g pMECS or pHEN 4 respectively. (If the expression vector is without a tag, the VHH fragment is further re-cloned into an expression vector with a tag (e.g. pHEN6) for easy purification afterwards). Bacteria cells are then transformed with the nanobody gene repertoire in the expression vectors to obtain a nanobody library (Hmila *et al.,* 2010). The nanobody library is super-infected with helper phages. Biopanning method is used to enrich for specific nanobodies which are attached on virions. Phage enrichment is achieved by several rounds of *in vitro* selection on the antigen of choice coated on microtiter plates or immunotubes. Phages are eluted with alkaline agent. The eluted phages are further used to infect *E. coli* cells, the bacteria cells are plated out to select each colony of the library containing an enriched nanobody gene. Viable

colonies are picked and expressed to generate periplasmic extracts for an initial ELISA test for antigen recognition on the antigen of choice. The positive ELISA colonies are grown and expressed and periplasmic protein extracts are extracted. For the colonies that were ligated into vectors without tags, they are recloned into tagged vectors before large scale expression. Purification of periplasmic extract is done with immobilized metal affinity chromatography followed by gel chromatography. Once produced and purified, further testing can commence.

Overview of results from the generation of nanobodies targeting trypanosome proteins

In this work, an unbiased immunization approach to generate nanobodies by immunizing an alpaca with trypanosome total lysate protein was used. Using phage display and bio-panning techniques, a cross-reactive nanobody code named Nb392 was selected from a VHH library (Figure 2). On ELISA, Nb392 binds to lysates of *Trypanosoma evansi (T. evansi)*, *T. brucei*, *T. congolense* and *T. vivax* (Figure 3). Imunoblotting and immunofluorescence assays (IFA) revealed that Nb392 recognizes a protein in the flagella. Antigen identification by a novel nanobody immunoprecipitation of trypanosome proteins and subsequent mass spectrometry showed that Nb392 targets 73Kda paraflagela rod 1 (PFR1) protein. Two different RNAi mutants with defective PFR assembly (*PFR2*^{RNAi} and *KIF9B*^{RNAi}) (Demonchy *et al.,* 2009) were used to further confirm the binding of Nb392 to PFR proteins (Figure 4) (Obishakin *et al.,* 2014).

DISCUSSION

Raising heat stable antibody that recognizes an ideal diagnostic target is one of the main barriers to diagnosis of Animal African Trypanosomiasis and other tropical infectious diseases. Based on the heat stable features of nanobodies and the field condition of trypanosome endemic regions which are predominantly tropical, the use of nanobody technology opens a new horizon and looks promising, it should thus be strongly considered. Nb392 represents an additional tool to study PFR proteins and it shows the potential use of nanobody technology for diagnostic and other protein research purposes in trypanosomes, opening up a new dimension in immunodiagnostics.

Figures

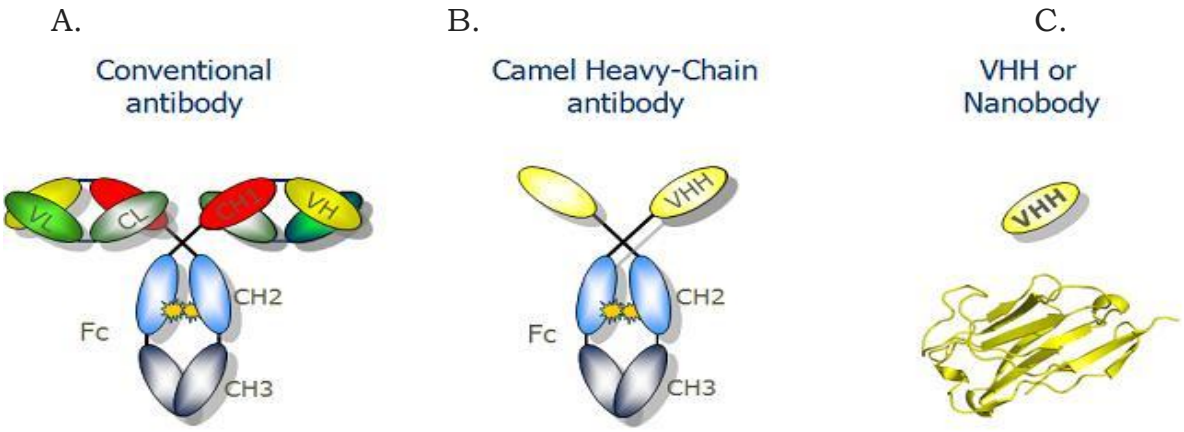


Figure 1. Conventional and VHH antibodies. Classical antibody (A), heavy-chain antibody (B), single-domain antigen-binding fragment (nanobody) derived from a heavy-chain antibody (C) (Muyldermans, 2009).

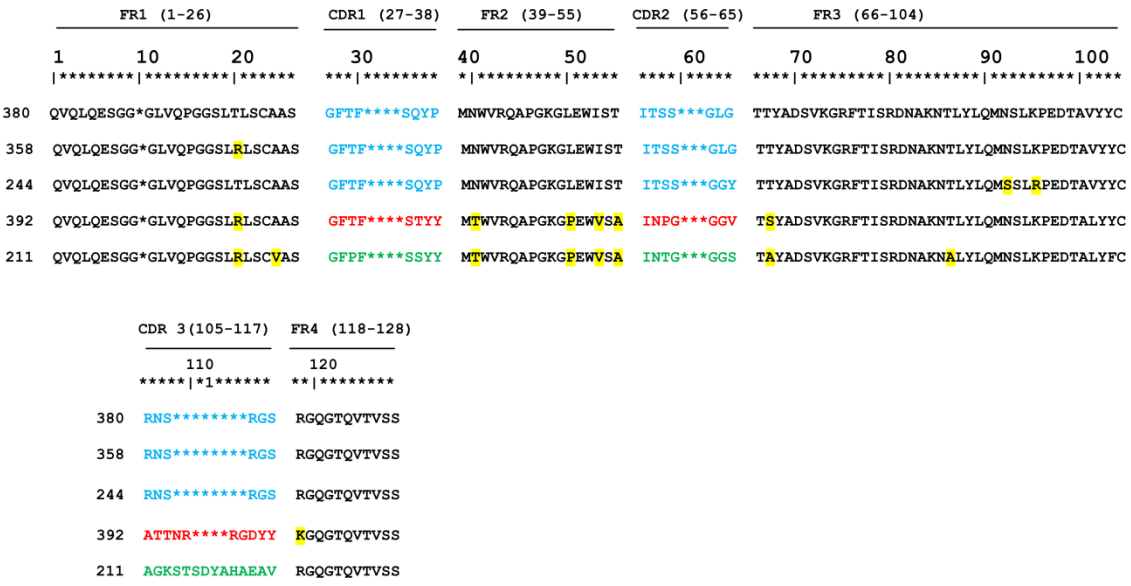


Figure 2. Sequence alignment of deduced amino acid sequences of the different cross-reactive nanobodies highlighting the framework (FR) and complementarity determining region CDR sequences. CDR sequences are highlighted in blue, red and green (corresponding to different families), while FR differences are marked in yellow. (Obishakin *et al.*, 2014).

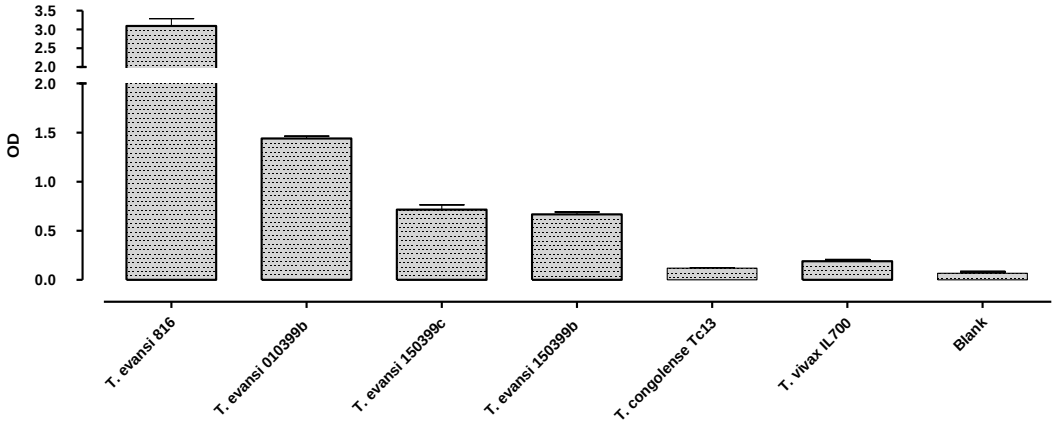


Figure 3. Solid-phase ELISA coating 5 µg/well of soluble protein of parasite lysates (*T. evansi* STIB 816, *T. evansi Itmas 010399b, T. evansi Itmas 150399c*, *T. evansi Itmas 150399b, T. vivax IL700* and *T. congolense* Tc13) and subsequent recognition by Nb392. Data represents results from three independent experiments performed in triplicates (±SD) (Obishakin, E., 2014).

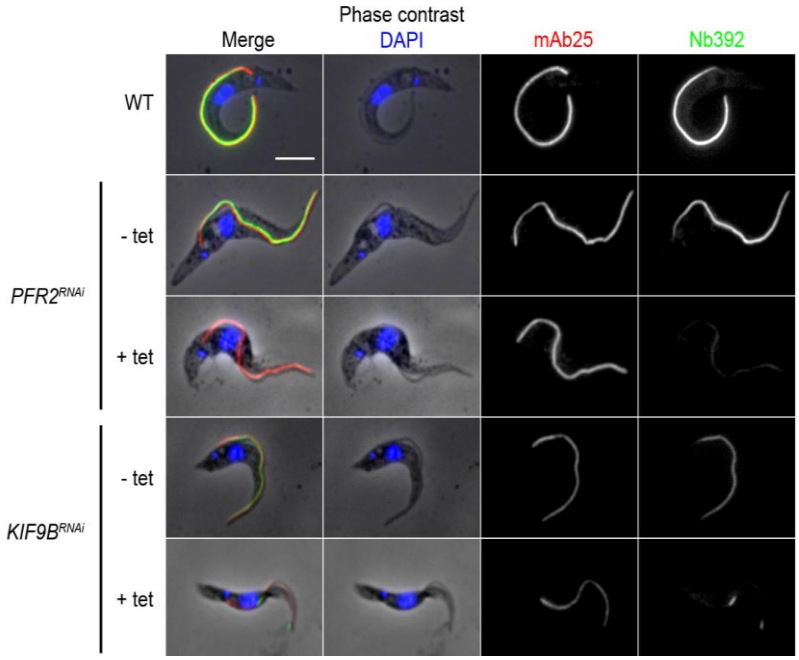


Figure 4. Immunofluorescence assay on *T.brucei* procyclic cells showing reactivity of Nb392 in two PFR mutants–Horizontal rows from top to bottom: Wild type (A) *PFR2*^{RNAi} uninduced (B), *PFR2*^{RNAi} induced (C), *KIF9B*^{RNAi} uninduced (D), *KIF9B*^{RNAi} induced (E), Vertical columns from left to right: combined phase-contrast DAPI, mAb25 and Nb392 images (left), DAPI images (middle left), mAb25 (middle right), Nb392 (right). Scale bar: 5 µm. (Obishakin, E., 2014).

AKNOWLEDGEMENT

I wish to acknowledge the Executive Director and the management of NVRI for the permission and support granted me for further training. I also wish to appreciate my mentor Professor Stefan Magez, the Belgian Government, the

Flemish Institute for Biotechnology (VIB) and the management of Vrije University Brussel for their funding supports.

REFERENCES

Deckers, N; Saerens, D; Kanobana, K; Conrath, K; Victor, B; Wernery, U; Vercruysse, J; Muyldermans, S; Dorny, P (2009). Nanobodies, a promising tool for species-specific diagnosis of taenia solium cysticercosis. *International Journal for Parasitology* 39: 625-633.

Demonchy, R; Blisnick, T; Deprez, C; Toutirais, G; Loussert, C; Marande, W; Grellier, P; Bastin, P; Kohl, L (2009). Kinesin 9 family members perform separate functions in the trypanosome flagellum. Journal of Cell Biology 187: 615-622.

Hmila, I; Saerens, D; Abderrazek, R.B; Vincke, C; Abidi, N; Benlasfar, Z; Govaert, J; El-Ayeb, M; Bouhaouala-Zahar, B; Muyldermans, S (2010). A bispecific nanobody to provide full protection against lethal scorpion envenoming. *The FASEB Journal* 24: 3479-3489.

Muyldermans, S (2013). Nanobodies: Natural single-domain antibodies. *Annual Review of Biochemistry*.

Muyldermans, S; Baral, T.N; Retamozzo, V.C; De Baetselier, P; De Genst, E; Kinne, J; Leonhardt, H; Magez, S; Nguyen, V.K; Revets, H; Rothbauer, U; Stijlemans, B; Tillib, S; Wernery, U; Wyns, L; Hassanzadeh-Ghassabeh, G; Saerens, D (2009). Camelid immunoglobulins and nanobody technology. *Veterinary Immunology and Immunopathology* 128: 178-183.

Obishakin, E., Stijlemans, B., Santi-Rocca, J., Vandenberghe, I., Devreese, B., Muldermans, S., Bastin P., Magez, S. (2014). Generation of a nanobody targeting the paraflagellar rod protein of trypanosomes, *PloS one*, *9*(12), e115893?

Van der Linden, R; Frenken, L; De Geus, B; Harmsen, M; Ruuls, R; Stok, W; De Ron, L; Wilson, S; Davis, P; Verrips, C (1999). Comparison of physical chemical properties of llama VHH antibody fragments and mouse monoclonal antibodies. Biochimica et Biophysica Acta (BBA)-*Protein Structure and Molecular Enzymology* 1431: 37-46.

Vaneycken, I; D'huyvetter, M; Hernot, S; De Vos, J; Xavier, C; Devoogdt, N; Caveliers, V; Lahoutte, T (2011). Immuno-imaging using nanobodies. *Current Opinion Biotechnology* 22: 877-881.

A STUDY ON HELMINTHIASIS OF CATTLE HERDS IN KACHIA GRAZING RESERVE (KGR) OF KADUNA STATE, NIGERIA

Henry .E. Nnabuife

Parasitology Division, National Veterinary Research Institute, Vom.

Email: ekennabusimple@yahoo.com

INTRODUCTION

Among all the livestock that make up the farm animals in Nigeria, ruminants, comprising sheep, goats and cattle, constitute the farm animals largely reared by farm families in the country's agricultural system (Lawal-Adebowale, 2012). Nigeria has population of 34.5million goats, 22.1million sheep and 13.9million cattle. Cattle represents a valuable asset in both traditional and modern agriculture, they provide meat, milk, skin and draught power. Additionally they may, in a traditional society be an essential part of the social system, representing a family wealth or they can be regarded as a survival kit by nomadic people (Fabiyi, 1984).

Despite its usefulness to man, cattle may be infected with pathogens that are dangerous to man and other domestic animals. Such are parasites which include helminthes of Gastrointestinal Tract (GIT). Helminth parasites come in three major classifications namely cestodes, nematodes and trematodes (Tibbo *et al*, 2011).

Helminth infections are important cause of production losses in livestock worldwide often necessitating antihelminthic treatment (Vercruysse and Clarebout, 2001). The significance of helminthiasis has been recognized by livestock farmers, right from the earliest of times and various methods have been employed by them to control helminthes in their animals including the use of medicinal plants and herbs and different grazing techniques (Bukhari and Sanyal 2011). The major control method employed against helminth parasites in developing countries as elsewhere is the use of chemotherapy (Aragaw *et al.*, 2010; Sargison, 2011).

The strategies for preventive control must involve stopping a build-up of large numbers of larvae on pasture and anticipation of periods for likely

multiplication of larvae on pasture and to move animals from such pastures during those periods. In order to achieve this goal there has to be a multifaceted approach involving grazing management, use of anthelmintics and dependence on acquisition of immunity.
Despite this importance, helminthes are the most neglected area of Veterinary care in the developing countries (including Nigeria) due to its chronic and insidious nature, with endemic pathogenic, vectors and diseases particularly where extensive grazing is practiced (Magona *et al.*, 2008; Bizimenyera *et al.*, 2008).

Grazing Reserves were established in Nigeria in major cattle producing areas to stabilize the pastoral production system, one of which is Kachia Grazing Reserve (KGR) in Kaduna State Nigeria. Monitoring cattle population and habitat conditions as well as ascertaining parasites and disease status are important component of herd health programme. Therefore, this study aims to determine the prevalence of gastrointestinal helminthes in cattle in KGR and to identify risk factors associated with helminthiasis in the study area.

MATERIALS AND METHODS

Study Area

The study was conducted in Kachia, a sedentary Fulani community of KGR in Kaduna state, Nigeria. The KGR comprises six (6) contiguous 'Blocks' containing 581 farmers and 5,252 people. Households per block ranges from 80 to 188 and a total of 23,327 cattle are owned by 569 households.

Sample size

Eighty-eight (88) households were selected randomly from 569 cattle-owning households. A total of 3,651 cattle were sampled and the sampling was spread over three (3) periods; Pre-intervention (1,525), Intervention (1,609) and Post-intervention (517).

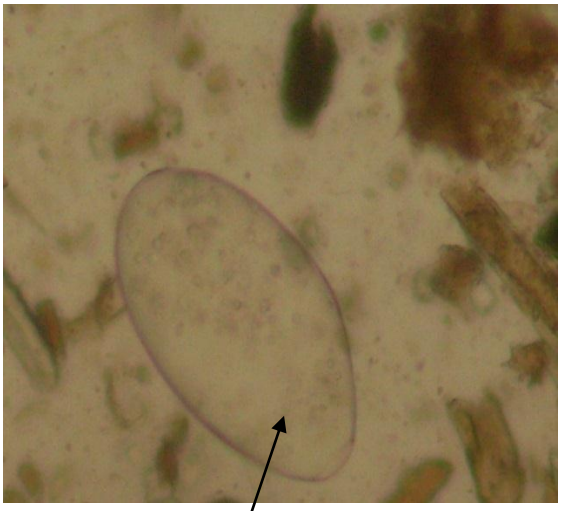
Collection and examination of faecal samples

Fresh faecal samples were collected per-rectum of cattle, labeled and kept in cool boxes before transporting them to the laboratory for further analysis. Faecal examination was by a quantitative floatation (McMaster technique) and

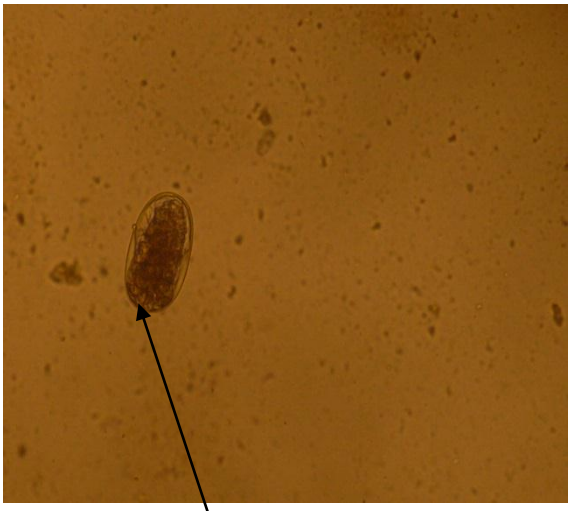
sedimentation method for detection of nematode, cestode and trematode eggs as described by [Hansen and Perry, 1990, Soulsby, 1982] respectively.

Data analysis

Data obtained was analyzed using Chi-Square (x^2) and Kruskal Wallis test [Sokal and Rohlf, 2001].



Egg of *Paramphistomum cervi*



Egg of *Oesophagostomum radiatum*

RESULTS AND DISCUSSION

Distribution and types of parasite eggs in the Pre-intervention, Intervention and Post-intervention periods

In March (Pre-intervention) 820 (53.77 %) were positive, intervention in June, 946 (58.79%) were positive and 205 (39.65%) were positive for post-intervention (Table 1).

Table 1: Intervention period-related prevalence of helminth parasites in cattle in KGR, Kachia

Period	Number examined	Number positive (%, infection rate)
Pre-intervention (March-April)	1,525	820(53.77)
Intervention (June-July)	1,609	946(58.79)
Post-intervention (Sept.-Oct.)	517	205(39.65)

Table 2: Distribution of parasites of cattle during the survey

Parasites	Pre-intervention Number infected (% infection rate)	Intervention Number infected (% infection rate)	Post-intervention Number infected (%infection rate)
Oesophagostomum radiatum	232 (17.90)	237(16.39)	57(23.08)
Bunostomum phlebotumum	112 (8.64)	106(7.33)	12(4.86)
Trichuris globulosa	189 (14.58)	213(14.73)	43(17.41)
Cooperia pectinita	93(7.18)	45(3.11)	3(1.21)
Monieza benedeni	9(0.69)	1(0.07)	0(0.00)
Toxocara vitulorum	7(0.54)	8(0.55)	0(0.00)
Strongyloides papillosus	76(5.86)	14(0.97)	1(0.40)
Strongylus langamus	1(0.08)	0(0.00)	0(0.00)
Schistosoma bovis	0(0.00)	2(0.14)	3(1.21)
Paramphistomum cervi	540(41.67)	736(50.90)	127(51.43)
Fasciola gigantic	37(2.86)	84(5.81)	1(0.40)

Table 3: Single and mixed helminth infections among cattle during the periods in KGR

Number of Parasites	Pre-intervention Number infected (%)	Intervention Number infected (%)	Post-intervention Number infected (%)
1	498 (32.66)	585 (36.36)	171 (33.08)
2	192 (12.59)	251 (15.60)	33 (6.38)
3	74 (4.85)	69 (4.29)	4 (0.77)
4	33 (2.16)	38 (2.36)	0 (0.00)
5	5 (0.33)	16 (0.99)	0 (0.00)
6	1 (0.07)	7 (0.44)	0 (0.00)

Table 4: Prevalence of helminth parasites in different age groups of cattle in KGR

Age group	**Pre-intervention**		**Intervention**		**Post-intervention**	
	Num. examined	Num. infec. (%)	Num. examined	Num. infec. (%)	Num. examined	Num. infec. (%)
<8month	101	39(2.56)	93	45(2.80)	27	3(0.58)
8mth-2yr	252	109(7.15)	256	117(7.27)	56	14(2.71)
>2yrs	1,172	672(44.07)	1,260	784(48.73)	434	188(36.36)
Total	**1,525**	**820 (53.77)**	**1,609**	**946 (58.79)**	**517**	**205 (39.65)**

The helminth eggs of Nematodes-*Oesophagostomum radiatum, Bonustonum phlebotomum, Tricuris globulosa, Toxocara vitulorum, Strongyloides papillous, Strongylus langamus;* Cestode-*Moneizia benedeni,* and Trematodes-*Schistosoma bovis, Paramphistomum cervi* and *Fasciola gigantica* were identified (Table-2). The range of parasites found in this present study was almost similar to that found in Benin City, Southern Nigeria (Edosomwan and Shoyemi, 2012).

Age and season played significant role in determining helminthes community species concentration in cattle in the KGR which is in agreement with the report of Fuentes *et al.* (2004).The result shows that age was a factor in the abundance of the parasites in the animals. Parasites were demonstrated more in adult animals than the young stock, and this could be due to the fact that older livestock may have been exposed more frequently to infective stages of the parasites. This is in agreement with the report by (Ibrahim *et al.*, 2008).The present study demonstrated that *P.cervi* was the most prevalent in all the periods followed by *O. radiatum* (Table 2).The high prevalence of *P.cervi* may be attributed to the favorable environmental factors of temperature and precipitation found in the KGR (Gupta *et al.,* 2004; Sukhdeo and Sukhdeo, 2004). Another possible factor is the preferential attention received by young stock compared to the adults by herdsmen who believed that the adult cattle have outgrown parasitic attacks

The significant effect of season on helminthes concentration in this study is evident in the parasite prevalence observed in the different periods. The higher prevalence observed during the intervention period could be ascribed to the fact that sample collection was done during the wet season. During this period, high moisture content increased development of larvae and abundant pasture, thus resulting in increased contact between the host and parasites. These findings are similar to Fakae (1990) and Bhattacharya and Ahmed (2005) who recorded higher incidence of parasitic infection during rainy season in Nigeria and India respectively. The study also revealed that the cattle in the study area were infected with single and mixed (multiple) infections. In addition to seasonal influence, the low post-intervention prevalence could be ascribed to the effectiveness of the deworming intervention of the animals using a broad-spectrum antihelmintic bolus containing a combination of 60% oxyclozanide and 40% levamisole hydrochloride (Kepxan Bolus, Kepro®, Holland).

CONCLUSION AND RECOMMENDATION

The results of this study indicate that helminthes of cattle are common in KGR. However, it was also observed that herd-herd treatment takes care of not only clinical cases, but subclinical ones too. It is recommended that farmers in KGR be enlightened on the need to treat the herds instead of treating individual cases. Creation of Grazing Reserve such as KGR for all the nomadic Fulanis will help to trace and effectively tackle the problems such as helminth infections.

ACKNWOLEDGEMENT

I am very grateful to the Executive Director and the Management of NVRI, Vom for the permission granted me to undergo the study.

REFERENCES

Aragaw, K., Teferi, M., Haile, A., &Tibbo, M. (2010). Effects of strategic helminthosis control on age at first lambing and lambing interval of communally grazed Menz ewes in Ethiopia. *Livestock Science*, 135(1): 38-43.

Bhattacharya, D.K. and Ahmed, K. (2005). Prevalence of helminthic infection in cattle and buffaloes. *India Veterinary Journal,* 82: 900 -901

Bizimenyera, E.S., Swan, G.E., Eloff T.N., Meyer, S. and Naidoo, V. (2008). Efficacy of Peltophorum affricanum sond (fabaceace) extracts on *Haemonthus contortus* and *Trichostrongylus colubriformis* in sheep. *Journal of Animal and Veterinary advances*, 7(4): 364-371.

Bukhari, S., and Sanyal, P. K. (2011). Epidemiological Intelligence for Grazing Management in Strategic Control of Parasitic Gastroenteritis in Small Ruminants in India – A Review, *Vet.World*, 4(2): 92-96.

Edosomwan, E. U. And Shoyemi, O.O. (2012). Prevalence of gastrointestinal helminth parasites of cattle and goats slaughtered at abattoirs in Benin City, Nigeria, *African Scientist. Vol.* 13(2): 109-114.

Fabiyi, J.P. (1984). Coping with parasitic disease problem in Nigeria. *Livestock Farmers Series* 4:13

Fakae, B. B (1990). The epidemiology of helminthosis in small ruminants under the traditional husbandry system in eastern Nigeria. *Veterinary Research Communication*, 14 (5): 381-391.

Fuentes ,M.V., Saez, S., Trelis, M., Galan-Puchades ,M.T., Esteban, J.G,(2004) The helminth community of the wood mouse, Apodemussylvaticus , in the Sierra Espuna, Murda, Spain , *Journal of Helminthology*, 78(3):219-223.

Gupta, B .V.Parshad, S.Giraya (2004). Maturation of *Paramphistomum cervi* in sheep in India. *Veterinary Parasitology*, 15:239-245.

Hansen, J. and Perry, B. (1990) The Epidemiology, Diagnosis and Control of Gastro-intestinal Parasites of Ruminants in Africa, A Handbook, (ILRAD) Nairobi, Kenya, pp 25-50.

Ibrahim, M.M., Al Ghamdi, M.A., Al Ghamdi and M.S. (2008). Helminthes community of Veterinary Importance of Livestock in Relation to Some Ecological and Biological

Lawal-Adebowale, O. A., (2012). *Intech- Dynamics of Ruminant livestock management in the context of the Nigerian Agricultural System* 4: 61-80.

Magona J.W., Walubengo J. and Odimin J.T (2008). Acute haemorrhagic syndronae of bovine trypanosomoses in Uganda. *Acta Tropica,* 107(2): 186-191.

Sargison, N. D. (2011). Pharmaceutical Control of EndoparasiticHelminth Infections in Sheep. *Veterinary Clinics of North America: Food Animal Practice,* 27(1): 139-156.

Sokal R.R. and Rohlf F.J (2001). *Biometry* Freeman Publishers, 887pp

Soulsby E.J.W (1982). *Helminths, Arthropods and Protozoa of Domesticated Animals,* 7th Edn Lea and Febiger, Philadelphia: Bailliere Tindall, London, pp. 212-258.

Sukhdeo, M., and Sukhdeo, S (2004). Trematode behavior and perceptual worlds of parasites, *Canadian Journal of Zoology*, 82: 292-315

Tibbo, M., Aragaw, K., Teferi, M., & Haile, A (2011). Effect of strategic helminthosis control on mortality of communally grazed Menz lambs of small holders in the cool central Ethiopian highlands, *Small Ruminant Research* 90(1-3), 58-63.

Vercruysse, J. and Clarebout, E. (2001). Treatment vs. non-treatment of helminth infections in cattle: Defining the thresholds. *Veterinary Parasitology, 98: 195-214.*

THERAPEUTIC AND SAFETY EVALUATION OF COMBINED AQUEOUS EXTRACTS OF *AZADIRACHTA INDICA* AND *KHAYA SENEGALENSIS* IN CHICKENS EXPERIMENTALLY INFECTED WITH EIMERIA OOCYSTS

Dapuliga C. C.[1a], Viyoff V. Z.[1b], Ozoani H. A.[1c], Aladelokun O. B.[1d] Onyiche T. E.[1], Ochibgo G. O.[1e]

[1]National Veterinary Research Institute, Vom, Nigeria
[a]Microbiology Department, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana
[b]Department of Epidemiology, University of Buea, Cameroon.
[c]Department of Medical Laboratory science, River State University of Science and Technology, Port Harcourt, Nigeria.
[d]Department of Biochemistry, University of Ibadan, Nigeria
[e]Department of Veterinary Physiology, Pharmacology and Biochemistry, University of Ibadan, Nigeria

INTRODUCTION

Coccidiosis is a major parasitic disease of poultry with significant economic impact (William, 1998). Anticoccidial drugs are commonly used to prevent and treat coccidiosis. However, indiscriminate use of anticoccidial drugs has led to the emergence of drug resistant parasites and presence of residual drugs in chicken products raising concerns about public health and food safety (Chapman, 1997; Orengo *et al.*, 2012). Anticoccidial vaccines are an alternative means to prevent coccidiosis, but efficacy, safety and cost effectiveness are still challenges for use in poultry (Sharma *et al.*, 2010). Consumers and poultry farmers around the world have voiced concerns about the use of present anticoccidial agents (Yang *et al.*, 2015). Therefore, there is a need for an alternative approach to prevent and treat avian coccidiosis necessitating an examination of the potential of natural products from plant extracts.
Azadirachta indica (AI) and *Khaya Senegalensis* (KS) both belonging to the Family Meliaceae have been reported to possess anticoccidial properties and have been used individually to combat avian coccidiosis. This property has been demonstrated by their ability to reduce oocyst count (Biu *et al.*, 2006; Nwosu *et al.*, 2012), inhibit inflammation (Ishaq *et al.*, 2014), and enhance

erythropoiesis (Iyare and Obaji, 2014; Sanni *et al.*, 2005).
Tipu et al., (2006) showed that combinations of herbs used against coccidiosis are effective and an economical alternative for prophylactic anticoccidial medication. Therefore, this study aimed to evaluate the therapeutic efficacy of the combined aqueous extracts of leaves of *Azadirachta indica* and stem bark of *Khaya senegalensis* in chickens experimentally infected with Eimeria oocysts.

METHODOLOGY

The stem bark of *Khaya senegalensis* and leaves of *Azadirachta indica* were collected from the environs of the National Veterinary Research Institute (NVRI), Vom-Nigeria. The plants were identified and authenticated in the herbarium at Federal College of Forestry, Jos, Nigeria and assigned voucher numbers FHJ 198 and FHJ199 for *Azadirachta indica* and *Khaya Senegalensis* respectively. Dried plant samples were ground into powder and macerated with distilled water for 72 hours. At the end of the extraction, the mixture was sieved and filtered. The filtrate was concentrated by drying in the oven at 40^{o}C. The dried extracts were stored at 4˚C until needed.
The extracts were screened individually to detect the presence of some phytochemicals according to the methods described by Trease and Evans, (1989).
Mixed *Eimeria* oocyst suspension (*Eimeria tenella*, *E. necatrix* and *E. brunetti*) was obtained from the Parasitology Division of the National Veterinary Research Institute (NVRI). Apparently healthy day old broiler chicks were obtained from a hatchery in Jos, Nigeria and reared for three weeks before commencement of the study.
In the efficacy studies, infected birds were monitored by daily collection and screening of faeces for oocysts presence and count was carried out. After establishment of the infection (7 days post inoculation), treatment commenced by oral gavage of the extract. The combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* was administered at a dose ratio of 1:1 at 100mg/kg, 200mg/kg, and 400mg/kg. The experiment included two control groups; negative and positive treated with distilled water and amprolium (Amprolium 250 WSP, Kepro (R) B.V Holland) respectively. All treatments lasted for five (5) days. Hematological and serum analysis as well as histopathological and oxidative stress examinations were carried out.
In the safety studies, the combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* was administered at the dose ratio of 1:1 at 100mg/kg,

200mg/kg, and 400mg/kg for 5 days while the control group received distilled water. Serum biochemical analysis, liver oxidative stress assays and histopathological examination was conducted.

All experiments were conducted in accordance with the principles and guide for the care and use of laboratory animals (NRC, 1996) and approved by the animal ethics committee of NVRI, Vom.

Data obtained from the study were summarized as Means ± Standard Error of Mean and differences between the means determined at 5% level of significance using the one-way analysis of variance.

RESULTS

The yield of the plants was 4.90% for AI and 7.02% for KS. The phytochemical screening revealed the presence of tannins, saponins, cardiac glycosides and steroids in both extracts. In addition, alkaloids and flavonoids were detected in *Azadirachta indica* while resins, terpenes and anthraquinones were not detected in both extracts.

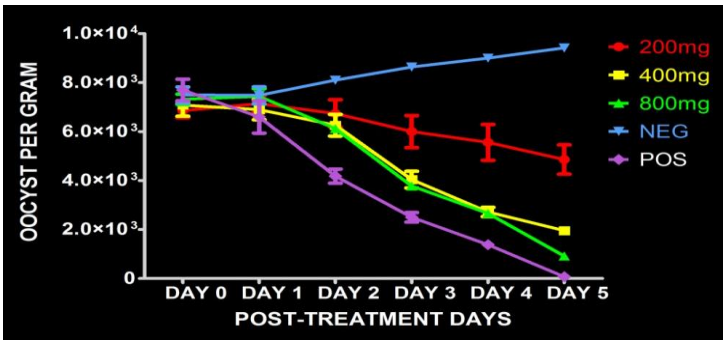


Figure 1: Effect of AI extract on Oocyst count

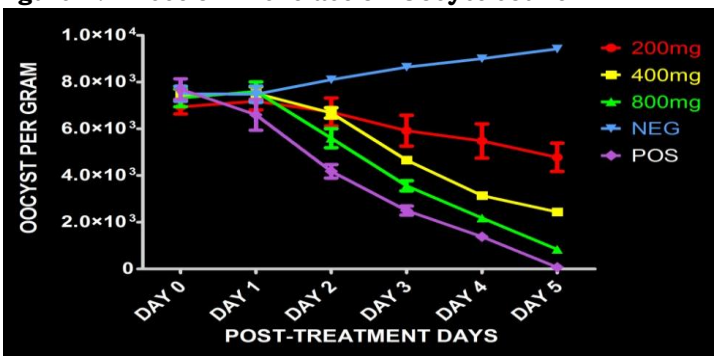


Figure 2: Effect of KS extract on Oocyst count

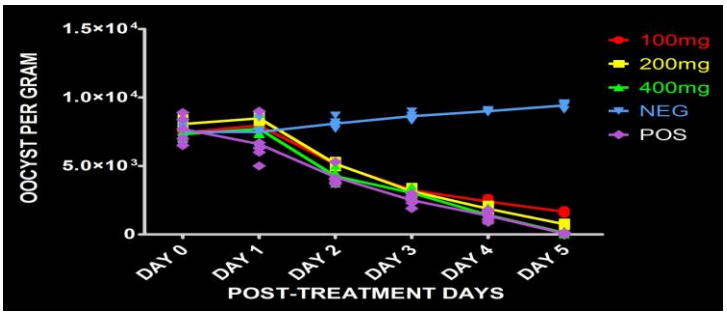


Figure 3: Effect of the Combined extracts on Oocyst count

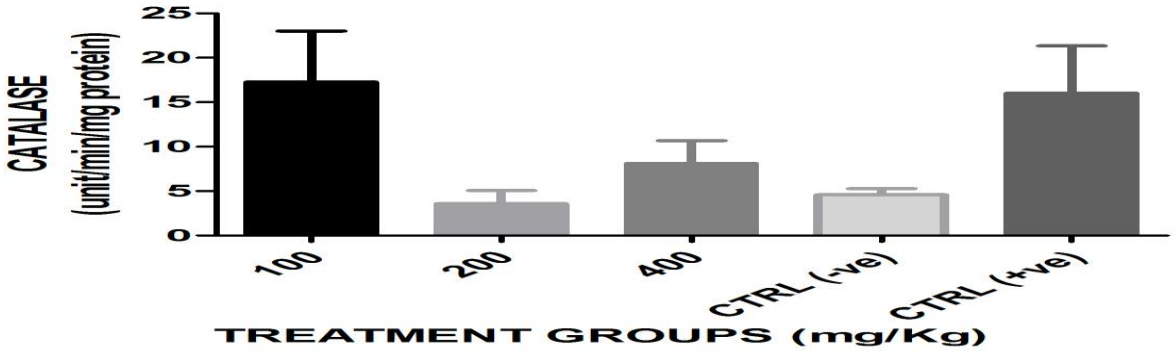


Fig. 4: Effect of Combined extracts of *Azadirachta indica* and *Khaya senegalensis* on Catalase Activity in the intestine of chickens infected with Eimeria oocysts

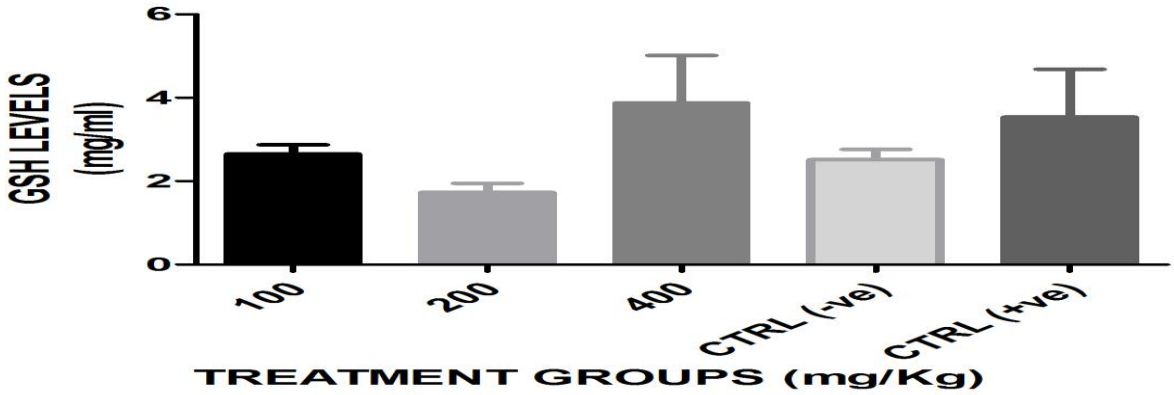


Fig. 5: Effect of Combined extracts of *Azadirachta indica* and *Khaya senegalensis* on Glutathione (GSH) levels of the intestine of chickens infected with Eimeria oocysts

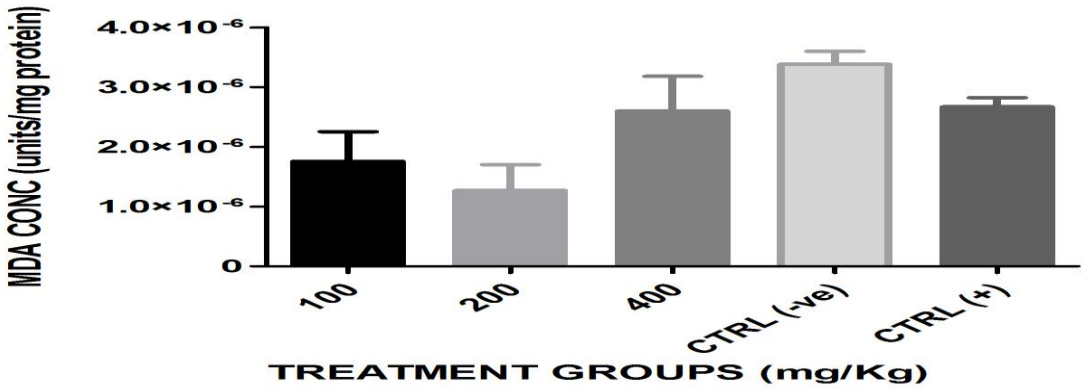


Fig. 6: Effect of Combined extracts of *Azadirachta indica* and *Khaya senegalensis* on lipid peroxidation (Malondialdehyde, MDA) of chickens infected with Eimeria oocysts

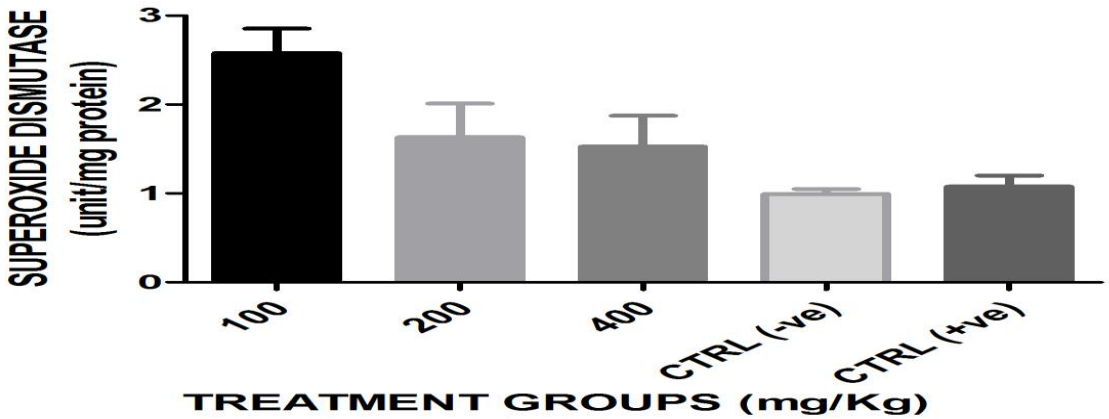


Fig. 7: Effect of Combined extracts of *Azadirachta indica* and *Khaya senegalensis* on Superoxide dismutase (SOD) activity of the intestine of chickens infected with Eimeria oocysts.

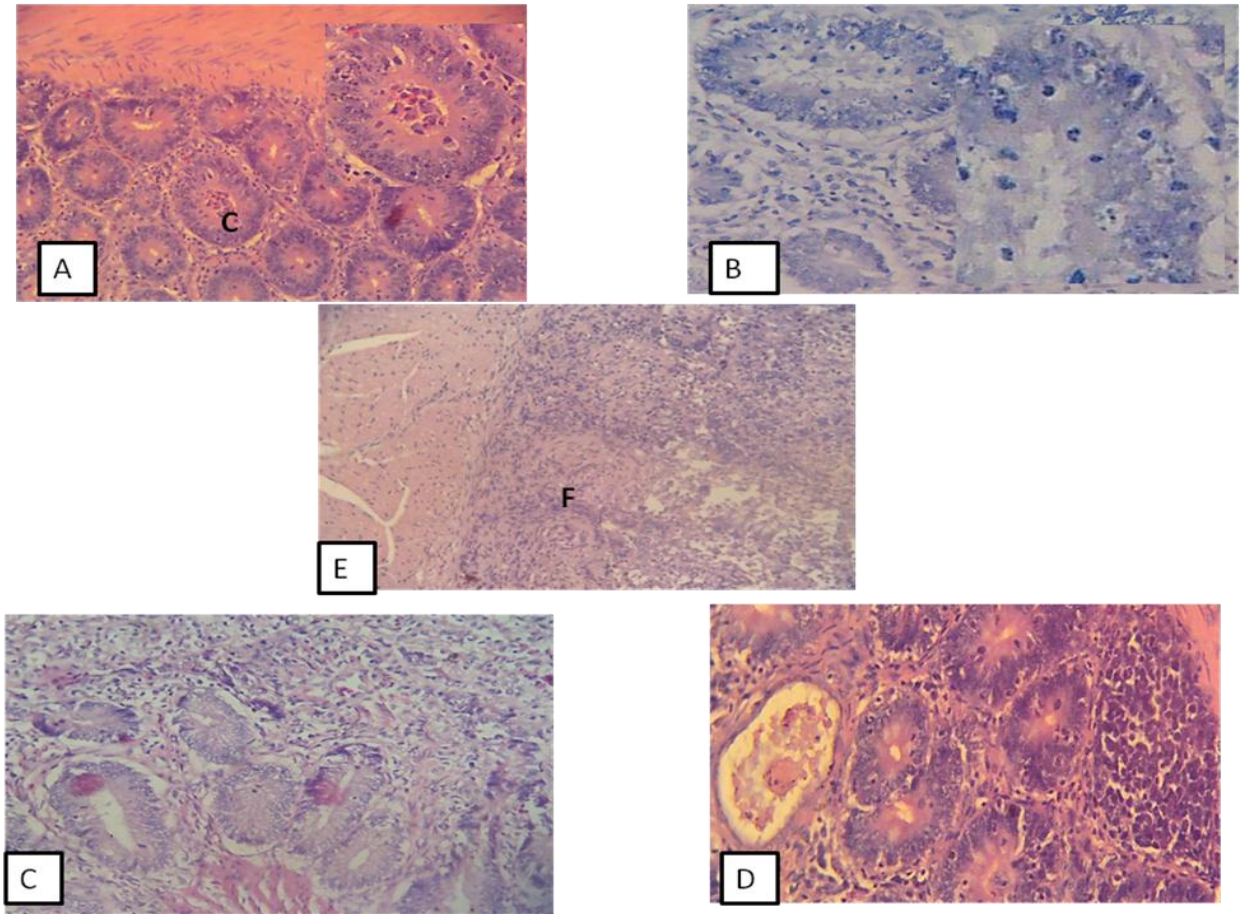


Fig. 8: Photomicrograph of the intestine of chickens infected with Eimeria oocysts and treated with graded doses of the combined extract and control

Plate A: 100mg/kg: Combined extract of KS &AI treatment; moderate cryptic destruction and intracryptic developmental stages of Eimeria H&E: X400

Plate B: 200 mg/kg: Intestine, infected with Eimeria spp; moderate cryptic destruction and intracryptic developmental stages of Eimeria and intestinal fibrosis H&E: X400

Plate C: Amprolium 10 mg/l: Chicken, Intestine, moderate cryptic destruction and ectasia, intracryptic developmental stages of Eimeria and intestinal fibrosis H&E: X400

Plate D: 400mg/kg extract: moderate cryptic destruction and intracryptic developmental stages of Eimeria H&E: X: 400

Plate E: Control (-ve) Intestine infected with Eimeria spp; severe cryptic destruction and intracryptic developmental stages of Eimeria and intestinal fibrosis, H&E: X400

Table 1: Liver function parameter of healthy chickens exposed to combined aqueous extract of *Azadirachta indica* and *Khaya senegalensis*

	Parameter						
Treatment	**ALT (U/L)**	**AST (U/L)**	**ALP (U/L)**	**TP (g/L)**	**TBIL (mg/dL)**	**DBIL (mg/dL)**	**ALB (mg/dL)**
Control	8.320 ± 0.489^{b}	21.32 ± 0.598^{b}	447.80 ± 7.055^{b}	35.32 ± 2.71^{b}	0.444 ± 0.005^{b}	0.233 ± 0.024^{b}	9.083 ± 1.124^{b}
100mg	7.560 ± 0.796^{b}	19.84 ± 0.379^{b}	420.70 ± 3.143^{a}	32.89 ± 1.459^{b}	0.406 ± 0.072^{b}	0.130 ± 0.017^{a}	14.16 ± 1.089^{a}
200mg	6.927 ± 0.058^{b}	18.89 ± 0.741^{a}	412.70 ± 6.374^{a}	26.51 ± 0.685^{a}	0.399 ± 0.032^{b}	0.087 ± 0.048^{a}	14.83 ± 0.724^{a}
400mg	5.600 ± 0.489^{a}	16.10 ± 2.330^{a}	312.40 ± 4.347^{a}	28.00 ± 0.907^{a}	0.358 ± 0.035^{a}	0.120 ± 0.032^{a}	13.88 ± 0.409^{a}

Values are expressed as mean ± S.E.M
Columns with different superscript shows significant difference ($p < 0.05$)

Table 2: Kidney function parameters of healthy chickens exposed to combined aqueous extract of *Azadirachta indica* and *Khaya senegalensis*

	Parameter			
Treatment	**CRE(mg/dl)**	**URE(mg/dl)**	**K(mEq/L)**	**Na(mEq/L)**
Control	0.418 ± 0.024^{b}	9.179 ± 0.540^{c}	5.613 ± 0.248^{a}	147.43 ± 1.003^{b}
100mg	0.288 ± 0.001^{a}	5.847 ± 0.540^{a}	5.013 ± 0.622^{a}	150.89 ± 1.028^{b}
200mg	0.285 ± 0.039^{a}	5.439 ± 0.490^{a}	5.403 ± 0.315^{a}	151.33 ± 4.290^{b}
400mg	0.285 ± 0.039^{a}	7.207 ± 0.544^{a}	5.957 ± 0.915^{a}	147.17 ± 0.301^{b}

Values are expressed as mean ± S.E.M
Rows with different superscript shows significant difference ($P < 0.05$)

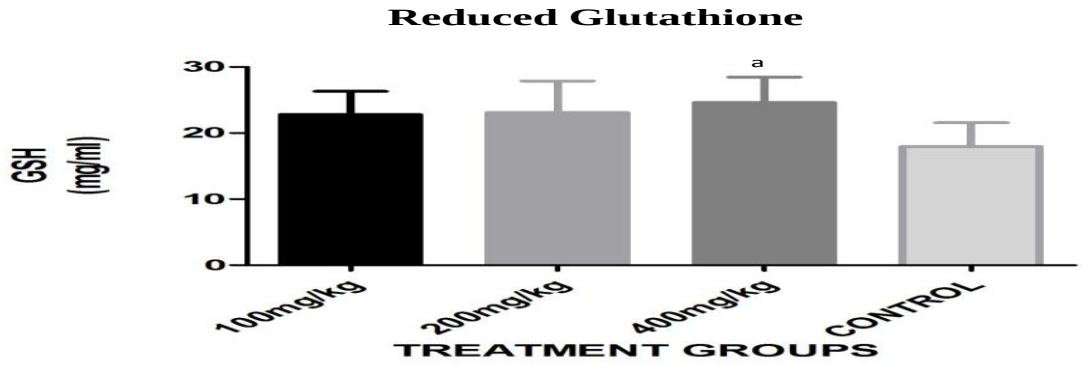


Fig 8: Effect of combined aqueous extracts of *Azadirachta indica* and *Khaya senegalensis* on reduced glutathione (GSH) levels of the liver of healthy chickens

Values are expressed as mean ± SEM where n=5

[a] Significant as compared with control ; ($p < 0.05$)

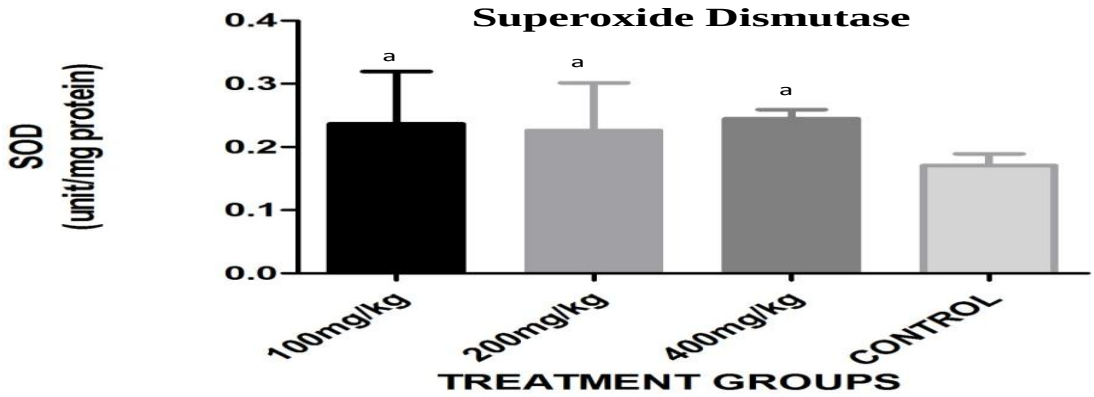


Fig 9: Effect of combined aqueous extracts of *Azadirachta indica* and *Khaya Senegalensis* on superoxide dismutase activity (SOD) of the liver of healthy chickens

Values are expressed as mean ± SEM where n=5

[a] significant as compared with control ; ($p < 0.05$)

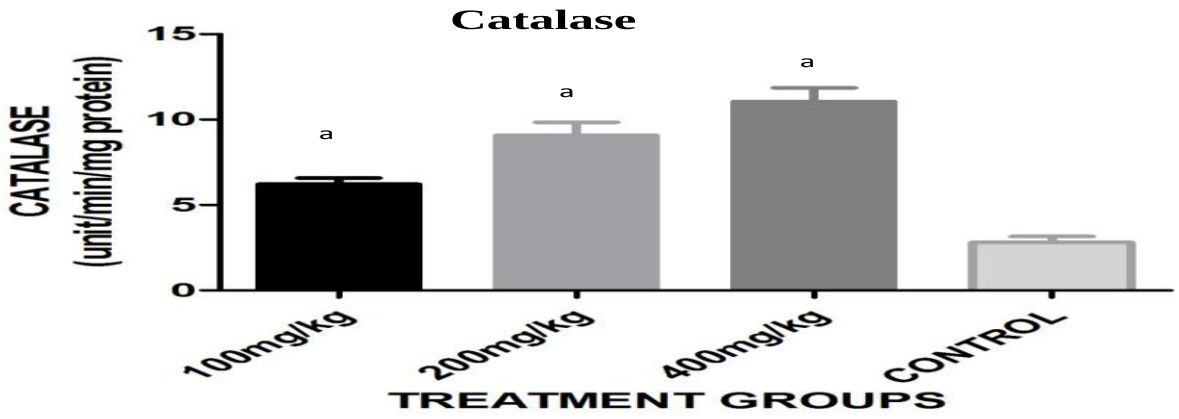


Fig 10: Effect of combined aqueous extracts of *Azadirachta indica* and *Khaya Senegalensis* on Catalase activity of the liver of healthy chickens
Values are expressed as mean ± SEM where n=5
[a] Significant as compared with control ; ($p < 0.05$)

CONCLUSION

This study shows novel findings with respect to the possible synergistic efficacy of the combination of aqueous extracts of *K. senegalensis* stem bark and *A. indica* leaves lending further credence to the folkloric use of these plants in the treatment of coccidiosis. The combination was safe at the doses and duration of administration and appeared to be protective to the liver and kidney.

RECOMMENDATION

The active phytocompounds in the extracts should be identified, isolated and characterized. Studies should be conducted using Molecular assays to explain the effect of the extracts on target regulatory genes involved in the disease pathogenesis. Other extraction solvents should be tried for possible enhanced activity.

ACKNOWLEDGEMENT

We wish to acknowledge the African Education Initiative (NEF), USA in partnership with the National Veterinary Research Institute, Vom Nigeria for providing grant for the research. We also thank all our mentors and Divisions that hosted the 2015 NEF Toxicology Internship Programme.

REFERENCES

Biu, A. A., Yusuf S. D., and Rabo J. S. (2006). Use of neem (*Azadirachta indica*) aqueous extract as a treatment for poultry coccidiosis in Borno State, Nigeria, *African Scientist*;7(3): 47 – 153.

Chapman, H.D. (1997). Biochemical, genetic and applied aspects of drug resistance in Eimeria parasites of the fowl, *Avian Pathology,* 26: 221–244.

Gararawa, J.U., Usman N.G., Ayi V.K., Alim H.M. (2011). Anticoccidial Resistance in Poultry: A Review, *New York Science Journal,* 4(8): 102-109.

Ishaq, F. N., Zezi A. U., and Olurishe T. O. (2014). *Khaya senegalensis* inhibits piroxicam mediated gastro-toxicity in wistar rats, *Avicenna Journal of Phytomedicine*; 4(6): 377 – 384.

Iyare, E.E. and Obaji N. N. (2014). "Effects of aqueous leaf extract of *Azadirachta indica* on some haematological parameters and blood glucose level in female rats," *Nigerian Journal of Experimental and Clinical Biosciences*; 2: 54 – 58.

National Research Council (1996). Guide for the care and use of laboratory animals, National Academy Press, Washington, D.C

Nwosu, C. U., Hassan S. W., Abubakar M. G. *et al.*, (2012). Anti-diarrhoeal and Toxicological studies of leaf extract of *Khaya Senegalensis*, *Journal of Pharmacology and Toxicology*; 7: 1–10.

Orengo, J., Buendia A.J., Ruiz-Ibanez M.R. *et al.*, (2012). Evaluating the efficacy of cinnamaldehyde and Echinacea purpurea plant extract in broilers against *Eimeria acervulina, Veterinary Parasitology,* 185: 158–163.

Sanni, F. S., Ibrahim S., Esievo K. A. N., *et al.*, (2005). Effect of oral administration of aqueous extract of *Khaya senegalensis* stem bark on phenylhydrazine-induced anaemia in rats, *Pakistan Journal of Biological Science*, 8: 255 – 258.

Sharma, P.A., Smith N.C., Wallach M.G *et al* (2010). Chasing the golden egg: vaccination against poultry coccidiosis, *Parasite Immunology,* 32: 590–598

Tipu, A. M., Akhtar M. S., Anjum M. I., *et al.*, (2006). New dimension of medicinal plants as animal feed, *Pakistan Veterinary Journal*, 26: 144–148

Trease, G. E. and Evans, M. D (1989). A Textbook of Pharmacognosy, Builler Tindall and Caussel London, 13th edn, Pp. 176-180

Williams, R.B (1998). Epidemiological aspects of the use of live anticoccidial vaccines for chickens, *International Journal for Parasitology,* 28: 1089–1098

Yang, W.C., Tien, Y.J., Chung, C.Y (2015). Effect of *Bidens pilosa* on infection and drug resistance of *Eimeria* in chickens, *Research in Veterinary Science,* 98: 74–81.

PUBLIC SERVICE REGULATIONS: A KEY TO ACHIEVING PRODUCTIVITY

Samme U. Andrew

Administration Division

INTRODUCTION

There are varied definition of the word administration by different Scholars and Writers. Augustus Adebayo in his book "Principles and Practice of Public Administration in Nigeria" define administration as the organization and direction of persons in order to accomplish a specified task; and in his words "when two men cooperate to roll a stone that neither could move alone, the rudiments of administration have appeared".

Based on the above, it is apt to state that an organization cannot exist without administration. In the National Veterinary Research Institute, the Executive Director/CEO has under him a hierarchy of subordinates, each with functions and responsibilities assigned for the purpose of achieving set objectives of the organization. The process requires planning, organizing, staffing, directing, coordinating, reporting and budgeting. These are the primary functions of an administrator commonly referred by the acronym POSDCORB.

The aim of this paper is to highlight some salient points, akin to public service regulation in Nigeria for enhanced productivity.

Protocols

The words Civil Service and Public Service are used interchangeably by different people. "The charter for the Public Service in Africa" adopted by the 3rd Biennial Pan-African Conference of Ministers of Civil Service, Winhoek, Namibia, 5th February, 2001 defines Public Service as "any Public entity that implements government policies and take decisions that affect the right of others or entities, be they individuals or not, and/or has a Public Service responsibility.

It has also been defined as "a body of professionals, full-time officials employed in the civil affairs or a state in non-political capacity" (Encyclopaedia Britannica, vol. 4 in F. E. Iyoha 1987:3).

The definition of the Civil Service or Public Service that is more encompassing is that which engulfs all adjuncts of the state that views Civil Service as "… a body of generalists and professionals, full-time workers employed by the National, State and Local Governments to help in the formulation and implementation of Public Policies or Laws" (Iyoha 1987:4).

"Public Service Employee" on the other hand is a person in Public employment given the responsibility for taking, implementation or enforcing decision within the public service regulation.

Public Service regulations determine the order in which the Public Service system can function and the principles and procedures governing its activity. Public service regulations provide the protection of government property in a wider sense by preventing abuse of office or power of authorities. Organizations are expected to operate in strict compliance with rules and where they do not, it brings about unhealthy tendencies in the organizations and in the society. Thus, the Public Service Rules may be regarded as the most important instrument of the Public Service system. All Public Servants must therefore submit themselves to the provisions of the rules and regulations as no one has the right to violate the rules of the Public Rules procedures.

As a key to achieving productivity, the Public Service Rules (PSR) lay down the measures of combating encroachments on the Public Service system, the existing rules of human and public relations together with the form and procedure on whish this is done. In other words, they determine the behaviour of Public Servants and regulate their most important human and social relations thereby exercising a substantial influence on the course of social development by way of formulating and implementing of government policies and programmes.

The Public Service Rules lie in their influence on Staff behaviour and on social relations. In any society law or rule performs a definite function of furthering the development of certain relations and obstructing the development of unhealthy tendencies.

The Public Service is a body or organ which enjoys continuity of existence. Its members unlike members of the National Assembly or a House of Assembly are not limited to a short term of office at the end of which they may or may not return to office. Elected members come and go but Public Servants remain in office.

Collectively, Public Servants command a pool of experience and know-how for formulating and implementing government policies and programmes. The Public Service is the instrument of the government of the day, but neither the service nor its members are the partisan of any particular political party.

A Public Servant is required to assist in formulating and implementing the policies approved by government whatever his personal or private opinions or attitudes may be. This does not mean that a Public Servant should undertake illegal action; if so directed, he should invite attention immediately to the legal position or requirement and advice on the proper action to take. He has the duty to also to advice on the implications of a policy or action.

The Public Service is indispensable irrespective of the type or regime, whether military or civilian. Under any political weather, it has to continue its traditional role of ensuring the orderly administration of Nigeria on continuous basis in the interest of political and social stability.

The administration of the country on continuous basis requires discipline and sanction. Rules and regulations must be adhered to and the interest of the service must be paramount. The Public Servant must, at all times, resist temptation from some quarters and uphold the principles of accountability, probity and transparency, national consciousness and the good image of the service.

Appointment

To be eligible for permanent and pensionable appointment into the Federal Public Service, the appointee must be a Nigerian as defined in the Constitution of the Federal Republic of Nigeria. In addition, the applicant must:

i. Possess a Certificate signed by the Chairman or Secretary of his Local Government indicating his State of Origin and Local Government.

ii. Not less than 18 years or more than 50 years of age.

iii. Possess minimum qualifications as specified in the approved Scheme of Service.

iv. Be certified by a Government Medical Officer as medically fit.

v. Possess a testimonial of good conduct from his last employer or if not previously employed, from the last School or Institution attended.

vi. State whether or not he has been convicted of a criminal offence.

vii. State all employment he was engaged in and, if he had left any employment, why he did so or if he is still in any employment whether or not he is under any obligation to remain in it.

viii. State whether or not he is free, from financial embarrassment, and

ix. Possess the NYSC discharge or exemption certificate where applicable.

Promotion Guidelines in Research Institutions and Colleges

The following criteria shall form the basis for advancement in any of the cadres within the Institution:

i. Length of service and pertinent experience.

ii. Performance

iii. Qualification

iv. Publications (where applicable) and

v. Vacancies in the establishment

(a) Movement from one grade level to a higher level will not be effected in less than two (2) years except in exceptional cases.

(b) All promotions and advancements shall be subject to positive performance rating from supervising officers and satisfactory service records. Performance will be given the greatest weighting in determining advancement.

(c) The acquisition of additional qualifications may not form the basis for consideration for promotions, except that the performance rating of the office is found consistently good and given that there is an appropriate vacancy at the next higher level.

(d) All officers who fall within the field of selection for any promotion exercise shall be considered except those who are under disciplinary action. The minimum number of years that an officer must spend in a post being considered eligible for promotion shall be as follows:

CONMESS	**Number of Years in Post**
2 – 4	Minimum of 3 years
5 – 7	Minimum of 4 years

CONHESS & CONRAISS	**Number of Years in Post**
01 – 05	Minimum of 2 years
06 – 12	Minimum of 3 years
13 – 15	Minimum of 4 years

In the case of Research Officers, moving from Senior Research Officer (CONHESS 02 to CONHESS 9) to Principal Research Officer (CONHESS 03 – CONHESS 11), the candidate or Staff must fulfil the following conditions:

> By promotion of a qualified Senior Research Officer with a minimum of Masters Degree plus the required relevant publications after spending at least three years on the previous grade (i.e. Senior Research Officer (CONHESS 02 or CONHESS 09).

Discipline

According to PSR, it is the duty of every Officer to acquaint himself with the disciplinary rules and any other regulations in force. Responsibility for breaches of the provisions PSR lies on every Staff.

As a general rule, disciplinary proceedings are initiated because of an Officer's misconduct or general inefficiency, under the provisions of PSR.

Misconduct

Is defined as a specific act of wrong-doing or improper behaviour which is inimical to the image of the service and which can be investigated and proven. It can also lead to termination and retirement. It includes

(a) Scandalous conduct such as:
   i. Immoral behaviour
   ii. Unruly behaviour
   iii. Drunkenness
   iv. Assault
   v. Battery
   vi. Refusal to proceed on transfer or to accept posting
   vii. Habitual lateness to work
   viii. Deliberate delay in treating of official documents
   ix. Failure to keep records
   x. Unauthorized removal of public records
   xi. Dishonesty
   xii. Negligence
   xiii. Membership of cults
   xiv. Sleeping on duty
   xv. Improper dressing while on duty
   xvi. Hawking merchandise within office premises
   xvii. Refusal to take/carry out lawful instruction from superior officers
   xviii. Malingering
   xix. Insubordination
   xx. Discourteous behaviour to the Public

General Inefficiency

General inefficiency consists of a series of omissions or incompetence the cumulative effect of which shows that the Officer is not capable of discharging efficiently the duties of the Office he holds.

Serious Misconduct

Serious misconduct is a specific act of very serious wrong-doing and improper behaviour which is inimical to the image of the service and which can be investigated and proven, may lead to dismissal. These include:

(a) Falsification of records

(b) Suppression of records

(c) Withholding of files

(d) Conviction on a criminal charge (other than a minor traffic or sanitary offence or the like)

(e) False claims against Government Officials

(f) Bankruptcy/Serious financial embarrassment

(g) Unauthorized disclosure of official information

(h) Bribery

(i) Corruption

(j) Embezzlement

(k) Misappropriation

(l) Violation of Oath of Secrecy

(m) Action prejudicial to the security of the State

(n) Advance Fee Fraud (Criminal Code 419)

(o) Holding more than one full-time paid job

(p) Nepotism or any other form of preferential treatment

(q) Divided loyalty

(r) Sabotage

(s) Willful damage to Public property

(t) Sexual harassment, and

(u) Any other act unbecoming of a Public Officer

Penalties for Breaches of Disciplinary Rules

The following are disciplinary measures that may be taken or applied under the Public Service Rules:

1. Verbal warning
2. Written warning
3. Reprimand
4. Stick reprimand
5. Reduction in rank or demotion for specified period
6. Reduction in Salary
7. Withholding or deferment of increment for a specified period
8. Loss of pay/non-payment for the corresponding days an Officer is not at duty post/station without authority
9. Stoppage of salary
10. Surcharge
11. Interdiction
12. Suspension
13. Termination of appointment
14. Dismissal from the Service

Disciplinary sanctions and penalties are many and are varied. Liability for breaches of service discipline is necessary to force the subordination of the personnel in the service for the proper and effective administration.

Interdiction

When a serious case that may lead to dismissal has been instituted against an Officer, such Officer may be placed on interdiction on not more than half pay pending the final determination of the case.

Suspension

Suspension is applied where a prima facie case, the nature of which is serious, has been established against an Officer and it is considered necessary in the public interest that he should forthwith be prohibited from carrying on his duties; pending the final determination of the case, the suspended Officer will cease to exercise the powers and functions of his Office and the enjoyment of his salary.

Dismissal

It must be noted that the ultimate penalty for serious misconduct is dismissal. An Officer who is dismissed from the service forfeits all claims to retiring benefits, leave or transport grant, etc.

Dismissal for breach of disciplinary rules is only permitted in cases stipulated in the Public Service Rules on serious misconduct. Any Officer who absents himself from duty or from Nigeria without leave renders himself liable to be dismissed from the service and, of course, the onus rests on him, to show that the circumstances do not justify the imposition of the full penalty. Again, an Officer involved in the conduct prejudicial to the security of the State may be dismissed from the service without following the normal procedure.

It is important to distinguish between dismissal and termination of appointment. Dismissal and termination are considered as punishment for misconduct. An officer who is dismissed or whose appointment is terminated solely for misconduct forfeits all rights and privileges such as leave, retiring benefits, transport, etc. An Office whose appointment is terminated for inefficiency does not forfeit them, but his retiring benefits may be reduced or entirely withheld if he also misconducted himself. That is say, if an Officer's services are terminated solely for misconduct, this is the equivalent of dismissal.

It should also be noted that if an Officer is convicted on a criminal charge, the officer may thereupon be dismissed without any of the procedures prescribed in the PSR being followed.

Removal of Temporary Staff

The employment of temporary Staff may be terminated provided such Staff has been informed on the grounds on which it is proposed to terminate his appointment and has been given and opportunity to submit representation, why he should not be terminated. Similarly, prior to the termination of his

service for inefficiency, such Staff should be warned of his failings and be given an opportunity to improve or to offer a satisfactory explanation of his failure to perform his duties efficiently.

Termination of Appointment during Probationary Period

If within his probationary period, it is established to the satisfaction of the authority empowered to appoint an Officer that he is not qualified for efficient service, his appointment may be terminated by that authority at any time without any further compensation than free transport to the place from which he was engaged; and such free transport will be granted only if his conduct has been good. If the termination is not due to misconduct, a month's notice will be given and leave may be granted if the Officer is eligible.

The appointment of an Officer on probation, who fails to secure confirmation in the pensionable establishment at the expiration of his probationary period, including such extension thereof as prescribed under PSR, may be terminated in the manner specific in PSR.

Retirement in Public Interest

Any Officer may be required to compulsorily retire from the Service on grounds which cannot be suitably dealt with by the disciplinary procedures and it is desirable in the public interest to do so. In every such case the question of pension and gratuity will be dealt with under the Pension Act for the time being in force.

Retiring Benefits

An Officer or employee who is dismissed or whose appointment is terminated for misconduct is not eligible to receive any retiring benefits. However, termination of appointment for inefficiency or in the public interest does not necessarily disqualify an Officer or employee from the award of retiring benefits. He is "retired" and, if otherwise eligible, may receive retiring benefits.

Other Penalties

Reproof, reprimand and strict reprimand mean censure of the Staff's breach of the provisions of PSR by the Management and do not entail any other negative consequences for the Staff.

Demotion is a heavier penalty and combines censure of the fault with a temporary reduction of earnings. The demotion or reduction in rank must be a temporary measure and should be applied for not more than one year or even a shorter period of six months may also be possible. The Staff should be returned to his previous post when the period expires. Furthermore, the demotion must consist in actually giving the offender a lower paid job, that is the job which must be less responsible and less skill.

As a general rule, only one penalty may be imposed for each breach of PSR. The right of choice is the Management's and the degree of the penalty must correspond to the seriousness of the breach committed.

Applying and lifting disciplinary sanctions are laid-down in the PSR procedures. Disciplinary sanctions are an essential feature of the PSR and of course, rules cannot be effective unless it is enforceable and no rule can be enforceable unless there is a sanction for its breach.

Fair Dismissal

Dismissal of an employee can only be fair if the employer can show the reason for it as one of those listed in the rules and regulations, and furthermore can show that the Management acted reasonably in the circumstances in treating that reasons as sufficient to justify dismissing the employee. Such reason may include:

1. A reason related to the employee's capability or qualifications for the job.

2. A reason related to the employee's conduct.

3. Redundancy.

4. A legal duty or restriction on either the employer of the employee which prevents the employment being continued.

5. Some other substantial reasons which could justify the dismissal.

Of course, unfair or wrongful dismissal occurs when sufficient notice is not given. When practicable, three queries and warnings should be served on erring Officer before considering the option of dismissal. Subject of course, to

the gravity of misconduct and circumstances of each case. In judging cases, the relevant sections of PSR should be applied.

Remedies for Unfair Dismissal

There are three possible remedies for unfair dismissal:

a. Reinstatement, that is to say, the employee is to be treated in all respects as though the dismissal had not occurred.

b. Re-engagement, that it to say, the employee is to be re-employed but not necessarily in the same job or on the same terms and conditions of employment.
c. Compensation, that is to say, payment of just, fair and reasonable compensation for loss of career expectations.

Model Disciplinary Procedure

i. A disciplinary procedure should be fair, full and quick.

ii. Each Staff should know that what the procedure consists of, its rules and the offences which can lead to dismissal.

iii. The procedure should state who has power to dismiss.

iv. Before dismissal takes place, a previous warning should have been issued, because as forewarned is to be forearmed.

v. The degree of misconduct in any particular case should determine whether it is sufficient to justify dismissal without notice.

vi. The records of past performance should be critically examined in order to establish the case of deadwoodism, incompetence and ineffectiveness.

vii. It is necessary to ensure that no one is dismissed unfairly and that there is a defence if a dismissed employee complains to a higher authority.

Right of Appeal and Petitions

Every Officer has a right of appeal against the decisions of the appropriate authority through the appropriate channels. However, a former Officer who is dissatisfied or aggrieved as a result of any decision of the appropriate authority has the right of appeal to the Federal Service Commission.

Again, an Officer or a person who was formerly an Officer has right of appeal to the President against the decision of the Federal Public Service Commission. A petition should be submitted within the stipulated period of not more than six months.

Record of Outstanding Work

Disciplinary sanctions are not the sole possible way of influencing offenders against labour discipline. Labour discipline is ensured by a conscientious attitude to work.

CONCLUSION

The Administrators' Position within an establishment or organization is strategic and pivotal. It is true to say that the pace of administration has come to be clearly recognized in every sector of human endeavour, as being the keystone to the success and indeed to the very existence of an organization. Being concerned with the planning, supervision and control of the establishment with which it is involved, it is no overstatement to declare that "whatever may be the future, the science of administration will be an essential instrument of human welfare".

REFERENCES

Federal Republic of Nigeria Official Gazette; Public Service Rules (2008) Edition

Augustus Adebayo "Principles and Practice of Public Administration in Nigeria". "3rd Biennial Pan African Conference of Ministers of Civil Service" Winhoek Namibia 5th February, 2001.

Encyclopaedia Britannica Vol. 4 in F. E. Iyoha 1987:3

RELATIONSHIP BETWEEN EMOTIONAL INTELLIGENCE AND JOB SATISFACTION AMONG EMPLOYEES OF NATIONAL VETERINARY RESEARCH INSTITUTE, VOM

Mshellia Priscilla Swatdunio (B.Sc, MBA, CNA, AMNIM)

Bursary Department, Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute, P.M.B 02, Vom

Correspondence: swandy.banta@gmail.com, 2348035983950

INTRODUCTION

We may know someone who is incredibly bright but cannot seem to pull his/her life together. Some may be brilliant students who drop out of the University or incredibly intelligent workers who can't seem to get ahead in their organization. We often know from our interactions with them that they have a good or superior intelligence even though it doesn't seem to ensure success. This scenario has led scientists over time to realize that Intelligence Quotient (IQ) may not be enough requirements for success in life. This has led to the realization that there is another type of intelligence which is equally very relevant for success which has been termed 'Emotional Intelligence'.

Emotional intelligence (EI) as a concept has gained popularity since Goleman wrote the book entitled "Emotional Intelligence" in 1995. EI can be acclaimed to be of psychological background even though it has gradually formed close ties with key management areas. Goleman (1998) defined EI as "the capacity for recognizing our own feelings and those of others, for motivating ourselves and for managing emotions well in ourselves and in our relationships". EI has as one of its premises that emotional responses may be logically consistent or inconsistent with particular beliefs about emotion. Relatively pure emotional reactions such as instances of happiness or fear displayed early in infancy may involve relatively little cognition; these probably are best evaluated as adaptive or maladaptive. But as the person develops increasingly complex representation of situations, his or her emotional reactions may merge with more complex thoughts to develop such cognitively saturated emotions as guilt or regret. Moreover, the person may develop sophisticated internal modules

that include standards of emotional functioning. These emotional reactions and models can be assessed according to their logical consistency, and hence their 'intelligence' (Mayer & Salovey, 1995). Social emotional competencies have been adjudged to be important aspects of both performance effectiveness and measures of professional success. Research has shown that rational intelligence quotient is no longer considered a good predictor of how effective an employee will be at work and in life; rather it has been suggested that EI is a better predictor in determining employee's effectiveness.

According to Spector (1997), job satisfaction refers to the evaluation of the job in all its ramifications and its antecedents. Factors such as financial rewards, resources to get the job completed, interest, challenge, use of valued skills, variety, occupational prestige, autonomy, relation to co-workers and supervisors, involvement in decision making and comfort factors such as hours, physical environment and travel time. Emotional intelligence has been said to be linked with job satisfaction through creation of self motivation which results from understanding the influence of our emotions on the factors responsible for creation of job satisfaction (Wong & Law, 2002; Ayub & Rafif, 2011; Seyal & Afzaal, 2013).

METHODOLOGY

Population and Sampling

The population of the study consisted of staff of the Federal College of Veterinary and Medical Laboratory Technology (FCVMLT), National Veterinary Research Institute, Vom. These staff comprised mainly of scientists and support staff. At first step, the population was ascertained from the nominal roll of the organization having a total of 215 staff and an additional 30 volunteers from the institute. A sample size of 132 was arrived at using the Cochran (1977) sample size determination table assuming p = 0.50 and t = 1.96. The Schutte (1998) questionnaire was used for data collection on Emotional Intelligence. Job satisfaction was measured using a one item overall job satisfaction questionnaire. Copies of questionnaires were administered and retrieved from respondents within a time span of two weeks. Pearson's correlation analysis was used as a tool for the analysis. The SPSS software was used for processing data collected.

RESULTS AND DISCUSSION

This study has the following hypothesis stated in null form

Ho: Emotional Intelligence has no significant relationship with employee job satisfaction

The hypothesis was tested using Pearson's correlation analysis and the results are shown in the table below.

TABLE 1: Correlation results

		JS	EI
JS	Pearson Correlation	1	0.047
	Sig. (2-tailed)		0.597
	N	131	131
EI	Pearson Correlation	0.047	1
	Sig. (2-tailed)	0.597	
	N	131	131

Key:

JS = Job satisfaction

EI = Emotional intelligence

Source: *SPSS Software*

From the correlation results, the Pearson's correlation value (0.047) suggests a significant relationship between EI and Job satisfaction ($p<0.05$). This means that the null hypothesis is rejected. It shows that EI creates a feeling of satisfaction not necessarily because the job may be so enjoyable but because the employees find ways of coping with their jobs despite any challenges that may be faced on the job. The findings are consistent with those of Adeyemo (2007), Abraham (2000) & Goleman (1995).

Employees with high EI are more likely to have higher levels of job satisfaction because they are more adept at appraising and regulating their own emotions than employees with low EI. For example, employees with high EI may be better at identifying feelings of frustration and stress, then subsequently regulating those emotions to reduce stress. Employees with high EI are more

resilient because they are able to understand the causes of stress and develop strategies and perseverance to deal with the negative consequences of stress (Copper & Sawaf, 1997). Conversely, employees with low EI are likely to be less aware of their emotions and possess fewer abilities to cope with their emotions when faced with difficult situations, thereby increasing their levels of stress and decreasing their level of job satisfaction.

CONCLUSION

Based on the research findings the study concludes that Emotional intelligence has significant relationship with employee job satisfaction. The pearsons correlation tests revealed that EI has a significant relationship with job satisfaction. Employees that are emotionally intelligent are likely to be more satisfied with their jobs.

It is therefore recommended that National Veterinary Research Institute, Vom and other allied organizations should evaluate EI levels of their prospective employees during recruitment. This can be done by administration of EI tests and consideration of test scores as significant factors in making recruitment decisions. Also, since EI is much less fixed than IQ it can be developed over time. This is what Goleman refers to as 'maturity'. This can be achieved by training, coaching and feedback so that steady progress can be made in the organization. Employees should be encouraged to work on imbibing emotional competencies so that they can attain emotional maturation since this is helpful in self motivation towards satisfaction. Job satisfaction resulting from EI maturation is likely to lead to increased productivity and create a healthy work environment.

Finally the researcher recommends that more studies be carried out in this area. More organizations should be studied with larger sample sizes especially with regards to gender, physical ability and job specialization.

ACKNOWLEDGEMENT

I acknowledge my supervisor, Professor Bello Sabo of Department of Business Administration, Ahmadu Bello University Zaria, and staff of Federal College of Veterinary and Medical Laboratory Technology, Vom for participating in the study.

REFERENCES

Abraham, R. (2000). The role of job control as a moderator of emotional Dissonance and emotional intelligence: Outcome relationship. *Journal of Psychology*, 134(2):35-42

Adeyemo, D.A. (2007). Emotional intelligence and relationship with job satisfaction and organizational commitment of employees in public parastatals of Oyo state, Ibadan *Medwell Journals* 2(1): 6-11

Ayub, N. & Rafif, S. (2011): The relationship between Motivation and Job satisfaction. *Pakistan Business Review: 28*

Cochran, W.G. (1977). *Sampling Techniques (3rded)* New York: John Wiley & Sons.

Copper, R & Sawaf, A (1997). *Emotional intelligence in leadership and organization* (1st Ed), Perigee trade

Goleman, D. (1995). *Emotional Intelligence: Why it can matter more than IQ.* New York, Bantam.

Goleman, D. (1998). *Working with Emotional Intelligence*, New York, Bantam

Mayer, J.D. & Salovey, P. (1995).Emotional Intelligence and the Construction and Regulation of Feelings, *Applied and Preventive Psychology 4,* 197-208

Seyal, H.A. & Afzaal, T (2013).An Investigation of relationship among Emotional Intelligence, Organizational Commitment and Job Satisfaction, Evidence from Academics in Brunei Darusalam. *International Business Research,* 6(3), 217-228 http://dx.doi.org/10.5539/ibr.v6n3p217

Spector, P.E (1977). *Job satisfaction application, assessment, causes and consequences,* Thousand Oaks, CA: Sage

Wong, C.S. & Law, K.S (2002).The effects of leader and follower emotional intelligence on performance and attitude: An explanatory study,*The leadership quarterly, 13(3)*

HIGHLY PATHOGENIC AVIAN INFLUENZA IN NIGERIA: OVERVIEW OF 2015 OUTBREAKS

Ifende, V.I. [1], Meseko, C.A. [2], Odita, C.I. [3], Akanbi, B.O. [3], Moses, G.D.[3], James, A.S.[3], Barde, I.J[3], Rimfa, A.G. [3], Ugbe, D[3]., Shittu, I.A. [2], Gado D [2], Olawuyi, A [2]., Joannis, T.M. [2], Kumbish, P.R[3]., Muhammad, L.U. [1], Okewole, P.A[1,3], Ahmed M.S. [2], Shamaki D.[2]

[1]Veterinary Extension Research and Liaison Services, NVRI, Vom
[2]Regional Center for Animal Influenza and TADs, NVRI, Vom
[3]Central Diagnostic Division, NVRI, Vom

Corresponding author: isioma_rapu@yahoo.com

INTRODUCTION

Avian influenza (AI) is an important disease of zoonotic origin that has caused morbidity and mortality in domestic animals, wildlife and humans (Muzaffar *et al.*, 2006; Olsen *et al.*, 2006; Gauthier-Clerc *et al.*, 2007). It is known to be caused by type A viruses of the family Orthomyxoviridae, and they are classified by their hemagglutinin (HA) and neuraminidase (NA) surface glycoproteins. Highly Pathogenic Avian Influenza (HPAI) has caused respiratory disease and deaths in poultry and poultry handlers that were inappropriately exposed to aerosols generated from handling chickens (Oladokun *et al.*, 2012)

The first case of HPAI in Nigeria was in February 2006 in a commercial poultry farm in Northern Nigeria and the causal virus was the H5N1. The source of the initial outbreak in Nigeria is still not clear; international trade in birds or wild migratory birds could be responsible (Ducatez et al., 2006).

The Federal Government already had an emergency preparedness plan in place which to a great extent paved way for the initial control strategy. The implementation curbed the first introduction of the disease as outbreaks were last recorded in 2008. However, a resurgence of the disease occurred in January 2015.

This study presents a synopsis of the 2015 outbreaks in Nigeria, from records available in NVRI as a major participant in the diagnosis and control of HPAI in Nigeria.

Seminar presented 5th November, 2015 at NVRI Auditorium

MATERIALS AND METHODS

The index cases were simultaneously reported from Lagos and Kano in Janauary 2015. They composed of eight (8) dead birds received by NVRI, Vom from Kano state ministry of Agriculture on the 7th of January, 2015. They were from Sabon Gari Live Bird Market and a commercial poultry farm. Previous samples had been received from live bird market on the 5th of Janaury in Lagos. The carcasses were examined at post mortem at the Central Diagnostic laboratory and tissues sent to the Regional Avian influenza laboratory for confirmatory diagnosis.

Nucleic acid extraction was carried out on tissue homogenate using Qiagen RNA extraction kit according to manufacturer's instruction. Thereafter, one-step RT-PCR assay targeting the matrix gene (M-gene) was carried out using GeneAmp® Gold RNA PCR core kit (Applied Biosystems, Foster City, CA, USA). Virus isolation was also carried out on samples that were positive by RT-PCR. Further molecular characterization and gene sequencing was carried out at the FAO/OIE reference laboratory.

Records of data at the Epidemiology unit of the Central Diagnostic Division were also evaluated to better understand the epidemiology of the 2015 outbreak. Samples and records evaluated in this study covered the period from the first index case in January 2015 to September 30, 2015. Arc Map was used for mapping and data analysis.

RESULTS

Post mortem findings

The carcasses examined were characterized by severe multi-organ hyperemia and ecchymotic haemorrhages. The spleen, thymus and liver were visibly enlarged. The lesions in the commercial chicken carcasses were consistent with HPAI, while that of the indigenous chickens were not pathognomonic.

Virology

As at September 2015, more than 500 cases from over 1000 samples submitted were confirmed positive for Influenza A subtype H5N1 in 20 States

and the FCT. The virus was widely disseminated in Kano, Lagos, Bauchi, Plateau and still circulating in Rivers State as at the time of this analysis.

Samples that are positive by RT-PCR were also successfully isolated in chicken embryonated eggs within 48 hours.

Molecular sequencing

The complete gene sequence of the current HPAI H5N1 in Nigeria was determined. The gene sequence has been submitted to GISAID under accession number *EPI 556504* and *EPI 567299- EPI 567305*.

The topology of the phylogenetic tree of the HA gene demonstrated that the H5N1 virus from Nigeria *A/chicken/Nigeria/15VIR 339-2/2015* falls within genetic *clade 2.3.2.1c*. In particular, the HA gene clustered with H5 viruses from China in 2013 and with H5N1 virus isolated from a Canadian returnee from China. The similarities observed were 99.3 to 99.5% respectively (Monne *et al.*, 2015).

Epidemiology

In the current outbreak**,** Avian Influenza outbreak was first confirmed in Lagos State in a live bird market followed by commercial poultry farm in Kano. By the end of January 2015, 15 States were already confirmed positive for the disease. At the time of this report, 20 States and the FCT are positive for AI in the on-going outbreak.

The epidemic curve of the disease shows that the outbreaks reached its peak in February (Fig. 1) and has declined since then with a sustained disease condition presently, with Kano and Plateau States having the highest number of disease outbreaks (Fig. 2)

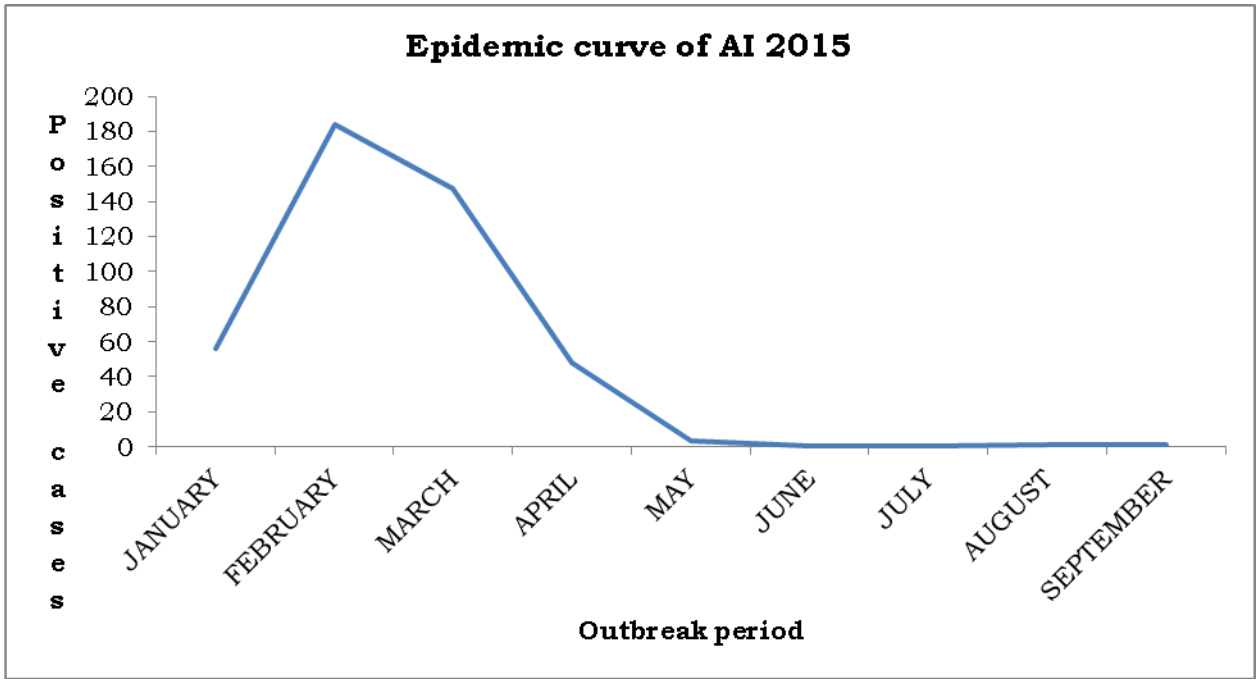


Figure 1: Graph showing HPAI positive cases per month

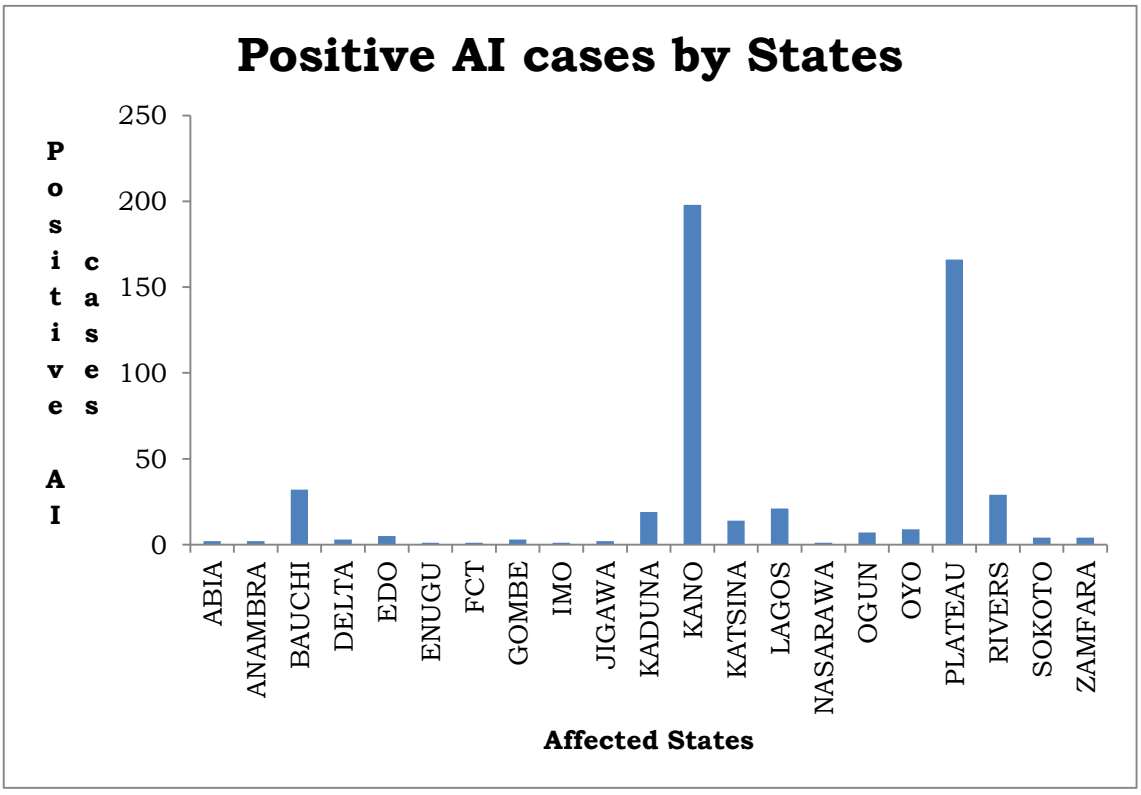


Figure 2: Graph showing HPAI positive cases per state

DISCUSSION

The resurgence of HPAI in 2015 since the last case in 2008 is a cause for concern. Molecular analysis revealed a new clade of H5N1 virus present in Nigeria in 2015, possibly introduced by migratory birds or trade in poultry.

The epidemic curve spiked in the month of February 2015, similar to 2006 and 2007 outbreaks respectively (Joannis et al., 2008). This may suggest a seasonal pattern for the virus that should be further investigated.

The results obtained provide evidence that a new clade of H5N1 virus has been introduced to Nigeria in 2015. A more prognostic approach and pragmatic plan is required for prevention and control of HPAI in Nigeria with this resurgence, in order to elude a likely endemicity of the disease.

ACKNOWLEDGEMENT

We like to also acknowledge the efforts of the following towards the diagnosis of HPAI;

Drs. Felix G.P., Makanju O., Chinyere C., Anefu E., Nwauda L., Mrs. Abiayi, E., Mrs. Chukwu, I., Mrs. Agama, Mrs. Liman F., Mr. Agada, O., Messers. Nyam D., Daspan A., T. Amos, J. Jonathan, R Akintola, B. Goma, L., Buba, M., Waje Atam S., Zal A., Dung G., Davou T.

Staff of VERLS and Poultry Divisions, FAO/OIE, and Federal Vetereinary Service

REFERENCES

Oladokun, AT; Meseko, CA; Ighodalo, E; Benshak, J; Ekong, PS (2012) Effect of intervention on the control of Highly Pathogenic Avian Influenza in Nigeria, *Pan African Medical Journal.*

Ducatez MF, Olinger CM, Owoade AA, De Landtsheer S, Ammerlaan W, Niesters HG, Osterhaus AD, Fouchier RA, Muller CP (2006). Avian flu: multiple introductions of H5N1 in Nigeria. *Nature* 442, 37

Gauthier-Clerc M, Lebarbenchon C and Thomas F (2007) Recent expansion of highly pathogenic avian influenza H5N1: a critical review. *Ibis* 149: 202-214.

Monne, I; Meseko, C; Joannis, T; Shittu, I; Ahmed, M; Tassoni, L; Fusaro, A; Cattoli, G (2015). Highly Pathogenic Avian Influenza A(H5N1) Virus in Poultry, Nigeria, 2015. *Emerg Infect Dis* 21.doi:10.3201/eid2107.150421.

Muzaffar, SB; Ydenberg, RC and Jones IL (2006) Avian Influenza: an ecological and evolutionary perspective for waterbird scientists, *Water birds* 29: 243-257

Olsen, B; Munster, VJ; Wallenstrom, J; Osterhaus, ADME; Fouchier, RAM; (2006). Global pattern of Influenza A virus in wildbirds, *Science* 312 (5772): 384–388.

Joannis, T M; Meseko, C A; Oladokun, A T; Ularamu, H G; Egbuji, A N; Solomon, P; Nyam, D C; Gado, D A; Luka, P; Ogedengbe, M E; Yakubu, M B; Tyem, AD; Akinyede, O; Shittu, A I; Sulaiman, L K; Owolodun, O A; Olawuyi, A K; Obishakin, E T; Fasina, F O (2008). Serologic and virologic surveillance of Avian Influenza in Nigeria, 2006-7, *Eurosurveillance* Vol 13, *Issue* 42

NVRI SEMINAR SERIES

GUIDELINES FOR PRESENTATION AND MANUSCRIPT PREPARATION

The NVRI Seminar Series is an edited publication of seminar presentations made at NVRI during the course of one calendar year. Seminar presentations provide a platform for research staff of the Institute and visiting scientists to present their research findings. The series is published annually.

Types of presentations

Research proposals, progress reports of on-going research, reports of completed studies and thesis are accepted for presentation. Reviews in any area of the Institute's mandate are made by invitation only.

Preparation for of manuscript for presentation

A Microsoft PowerPoint® presentation and an extended abstract are to be submitted to the secretary of the Committee one week before the presentation is due.

Preparation of manuscript

The manuscript should be written as an extended abstract with the following: Title of Paper, Author(s), Address including e-mail, Introduction, Methodology, Results and Discussion, Acknowledgements and References. Only one figure and one table will be accepted. The title of presentation should not be more than 15 words with the names of all authors and their affiliations. Only the email of the corresponding author and telephone numbers are required.

Duration of Presentation

The presentation usually lasts 20 minutes followed by another 20 minutes of discussion.

Check list for slide presentation

1. Visual Aid
   Please check that slides are:
   a. Not too many for a twenty-minute presentation
   b. Contents are coordinated with speech
   c. Slides are neat and spaced to fill the screen
   d. Simple and free from excessive data
   e. Free from garish colour and other embellishments that may distract from your message
   f. Have a balance of data, lists, and information with photographs interspersed throughout
2. Speaker
   Please ensure that

   a. You are familiar with speech and slides
   b. You are dressed properly to make your audience comfortable
   c. Reduce excessive gestures
   d. Avoid reading from the slides and notes all the time
   e. Shall keep to the time allocated for presentation